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CORPUS CAVERNOSUM ELECTROMYOGRAPHY IN THE DIAGNOSIS OF ERECTILE DYSFUNCTION

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CORPUS CAVERNOSUM ELECTROMYOGRAPHY IN THE DIAGNOSIS OF ERECTILE DYSFUNCTION

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dr. P.F.A. Mulders

学而不思则惘,思而不学则殆

孔子(公元前551--公元前479)

Learning without thinking leads to confusion; Thinking without learning ends in danger.

Confucius (551 BC – 479 BC)

Dedicate to my parents

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List of abbreviations

A	amplitude
ASIS	anterior superior iliac spine
AVSS	audiovisual sexual stimulation
CAI	cavernous arterial insufficiency
CC	corpus cavernosum
CC-EMG	corpus cavernosum electromyography
CSM	cavernous smooth muscle
CV	coefficient of variation
D	duration
DF	dominant frequency
DFP	number of dominant frequency periods
DM	diabetes mellitus
ED	erectile dysfunction
FFT	fast Fourier transformation
ICI	intracavernous injection
IP_3	inositol 1,4,5-triphosphate
NE	norepinephrine
PDE 5Is	phosphodiesterase type 5 inhibitors
PPDU	penile-pharmaco duplex ultrasonography
PV	propagation velocity
Rmax	maximum cross-correlation coefficient
Rmax-lon.	*Rmax* of longitudinal pairs
Rmax-bi.	*Rmax* of bilateral pairs
RRP	radical retropubic prostatectomy
SC	spinal cord
SMA	sympathetically mediated activity
SSR	sympathetic skin response
VOD	veno-occlusive dysfunction
VRF	vascular-risk factor

Chapter 1

Introduction and outline of the thesis

Erectile dysfunction (ED), defined as the consistent or recurrent inability to achieve and/or maintain a penile erection sufficient for sexual intercourse, affects more than 150 million men across the world [1]. The estimated prevalence in The Netherlands varies between 10 to 20% [2, 3]. As a result of basic science and clinical advances, the diagnosis and treatment of ED has undergone revolutionary improvement over the past two decades [4, 5]. Indeed, the true revolution in ED management occurred after the emergence of effective oral medications—phosphodiesterase type 5 inhibitors (PDE 5Is). On one hand, the majority of patients can be treated by primary care physicians without a complicated diagnostic procedure [4]; on the other hand, the number of ED patients who pursue a treatment more than doubled since then [5]. Among them, those who have no response or who have contraindications to PDE5Is need to be handled by specialists [4]. An etiological diagnosis is usually relevant in this group of patients, in order to avoid the selection of an improper treatment and to choose the right candidates for a more invasive therapy.

Penile flaccidity and erection are complex neurovascular processes. When the penis is flaccid, the cavernous smooth muscle (CSM) and the smooth muscle of the arteriolar and arterial walls are contracted mainly by the sympathetic discharge allowing only a small amount of arterial flow for nutritional purposes. To achieve an erection, the sympathetic activity is inhibited and parasympathetic pathway is activated, resulting in relaxation of the CSM, dilation of the arterioles and arteries, and increased intracavernous blood flow. Therefore, the integrity of penile vasculature, the CSM and the autonomic innervation are crucial for both penile erection and flaccidity [6].

Thanks to the inventions of pharmacotesting and penile-pharmaco duplex ultrasonography, the penile vasculature can be evaluated reliably in a minimally invasive way [4]. However, a non-invasive method to directly investigate the CSM and its autonomic innervation is still lacking. Penile biopsy has been introduced to evaluate the integrity of the cavernous tissue [7], however, to date its application is limited only in a niche of clinical and basic research, mainly due to its invasiveness and unspecific information obtained [8]. To evaluate neurogenic contributing factors, a variety of

neurological tests such as sacral evoked potentials, latency of the bubocavernous reflex, dorsal nerve conduction velocity, sympathetic skin response, etc. have been used [4, 9]. However, these methods either study the "wrong" nerve (somatic rather than autonomic nerve) or the "wrong" location of the "right" nerve, and therefore, the information obtained are unspecific, indirect, and unreliable [9]. Nowadays these methods are rarely applied by urologists.

This major deficit in the diagnosis of ED was firstly addressed by Wagner et al. in 1989 by proposing the registration of electrical activity originating from the corpus cavernosum [10]. Since then this method attracted much attentions and was intensively investigated by several investigators [9, 11-13]. The introduction of surface electrodes made it totally noninvasive and therefore highly acceptable for both patients and physicians [14]. A formal denomination, corpus cavernosum electromyography (CC-EMG), was coined in 1993 at the first standardization workshop. Similar to electrocardiography for cardiac muscle and electromyography for skeletal muscle, recording the electrical activity of the CSM to evaluate its function and innervation is logical. Studies in different institutions indicated that CC-EMG is able to diagnose myo- and neuropathy in patients with specific clinical conditions such as diabetes mellitus, radical prostatectomy and spinal cord lesions [9-14]. Moreover, it was shown that it may play a role in selecting candidates for invasive therapies [15]. Notwithstanding these promising finding, its clinical application was hindered by a series of technical and practical difficulties [16]. These difficulties include: (i) lack of standardization of the recording techniques as well as signal processing and signal analysis methods. (ii) doubts regarding the reproducibility of CC-potential. (iii) lack of objective and quantitative criteria to interpret CC-potentials. And (iiii) insufficient understanding of the electrophysiology of the CC and the recorded signals (CC-potentials).

The wish to further develop this promising method formed the starting point of this study, which was facilitated by the European Commission within the framework of the COST Action (B18) program.

In summary, the objective of this study was to develop CC-EMG into a useful clinical tool for evaluating the functional state of the CSM and its autonomic innervation. To accomplish this, the next steps were taken:

1. Standardization of the methodology of signal recording and signal processing (**Chapter 3**).
2. Establishing initial understanding of CC-potentials (**Chapter 4**).
3. Establishing a comprehensive, objective and versatile methodology for signal analysis (**Chapter 5**).
4. Assessing the reproducibility of the CC-EMG and the effect of potentially confounding factors of CC-EMG (**Chapter 6**).
5. Validation of parameters in well-defined clinical conditions (**Chapter 7**).

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Chapter 2

The Value of Corpus Cavernosum Electromyography in Erectile Dysfunction: Current Status and Future Prospect

Xiaogang Jiang, Tommy GW Speel, Gorm Wagner, Eric JH Meuleman, Hessel Wijkstra

Eur Urol 2003;43:211-218.

Abstract

In the last decade several investigators have tried to develop corpus cavernosum electromyography (CC-EMG) as a direct clinical method to evaluate the state of the penile autonomic innervation and the cavernous smooth muscle. Both basic and clinical studies have shown promising results. However, its application as a diagnostic tool with clinical relevance was hindered by insufficient knowledge of cavernous smooth muscle electrophysiology, lack of standardization, technical and practical difficulties and problems in the interpretation of the results. Recently the European Commission created the so-called COST Action B18 (corpus cavernosum EMG in erectile dysfunction), aiming to strengthen the coordination of the European research groups and give the development of CC-EMG a new impetus. This review presents an overview of the physiological background, the current status of CC-EMG, and discusses possibilities for further developments.

Keywords

Erectile dysfunction corpus cavernosum smooth muscle electromyography CC-EMG

Introduction

With the introduction of new diagnostic techniques in the last two decades, to date patients with erectile dysfunction (ED) can be diagnosed rather precisely, especially the endocrine and vasculogenic contributing factors. However, the cavernous autonomic innervation and the cavernous smooth muscle (CSM), which are essential for both penile erection and flaccidity, cannot be directly assessed. This major deficit in the diagnosis of ED was firstly addressed by Wagner et al. in 1989, who described the possibility of recording electrical activity originating from the corpus cavernosum, proposing that it might become a useful clinical method to evaluate the autonomic innervation of the penis [1]. A formal denomination, corpus cavernosum electromyography (CC-EMG), was coined in 1993 at the first standardization workshop. Over the past 13 years several research groups have shown scientific interest and made great effort to develop the method as a clinical useful tool. Quite a number of advances have been presented [2-5]. However, its application as a diagnostic tool with clinical relevance was hindered, by insufficient knowledge of the CSM electrophysiology, lack of standardization, technical and practical difficulties, and problems with the interpretation of the results. Being a possible direct and minimally invasive method to evaluate the CSM and its autonomic innervation, CC-EMG still seems to be of major importance to explore further. Initiated by an application of Wijkstra and Meuleman, in 2000, the European Commission created the so-called COST Action B18 (corpus cavernosum EMG in erectile dysfunction), aiming to strengthen the coordination of the European research groups and to give the development of CC-EMG a new impetus.

This review presents an overview of our current knowledge of cavernous electrophysiology, materials and methods of CC-EMG recording, clinical recordings and future developments.

Fundamental Knowledge

Physiological background

Both penile erection and flaccidity depend on the synchronized relaxation and contraction of the CSM cells, which are controlled by the autonomic innervation. Because the innervation of the CSM is relatively sparse and insufficient to account for the synchronous relaxation and contraction of all the CSM cells, direct intercellular communication in the responses is necessary. A pathway for such intercellular communication is provided by gap junctions between the CSM cells. Via gap junctions, second messenger molecules, ions and various metabolites can diffuse freely between the CSM cells. By this mechanism the CSM cells form a functional syncytium [6, 7].

The concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) is the key to the regulation of the CSM tone. An increase of $[Ca^{2+}]_i$ leads to contraction of the CSM cells, in turn, maintain the penis in flaccidity state; while relaxation, which leads to penile erection, is mediated by a decrease of $[Ca^{2+}]_i$ [7, 8] .

The mechanism of the contraction of the CSM is still not fully clarified. It is known that the flaccid state is maintained mainly by the release of norepinephrine (NE) from the sympathetic nerve fibres, in turn, activating specific α-adrenoceptor population, although the contributions of other agents, e.g. endothelins and prostanoids such as prostaglandin $F_{2\alpha,}$ cannot be excluded [7]. Both α_1- and α_2-adrenoceptors are present in human corpus cavernosum tissue, of which, α_1-adrenoceptors seems to be predominant functionally [7]. Stimulation of α_1-adrenoceptor forms inositol 1,4,5-triphosphate (IP_3), inducing Ca^{2+} release from the sarcoplasmatic reticulum (SR, intracellular compartment of Ca^{2+} storage). In the meantime, Ca^{2+} released from the SR can active Ca^{2+} -gated Cl^- channels, leading to an outward flow of Cl^- and depolarization of the cell membrane. Consequently, L-type voltage-gated Ca^{2+} channels (VGCCs) open, leading to Ca^{2+} influx from the extracellular space [8]. Activation of α_2-adrenoceptor may promote contraction of smooth muscles by increasing Ca^{2+} influx via opening of L-type VGCCs; meanwhile, an increase of IP_3 can be induced, leading to Ca^{2+} release from the intracellular stores [9] (Fig. 1).

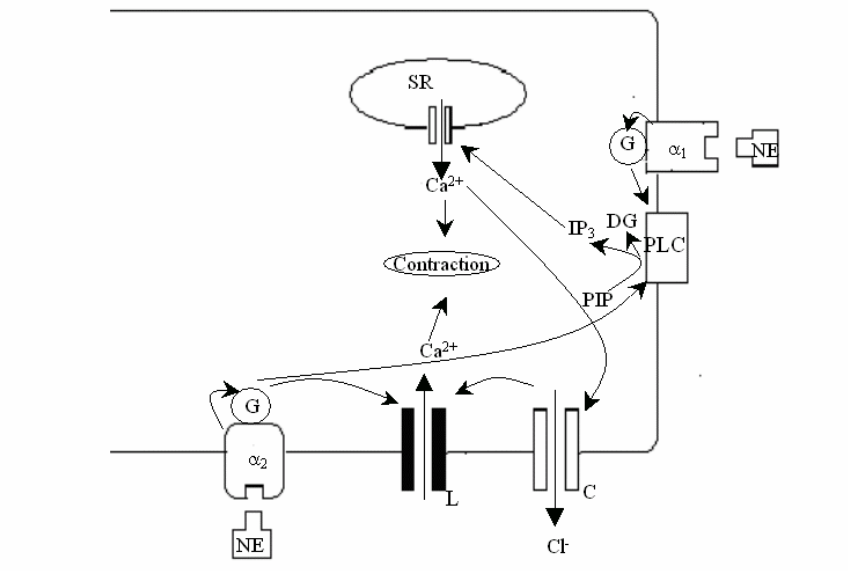

Figure 1 The mechanism of norepinephrine (NE) mediated contraction of the cavernous smooth muscle cell. Stimulation of α_1-adrenoceptor (α_1) mainly forms inositol 1,4,5-triphosphate (IP_3) inducing Ca^{2+} release from the sarcoplasmatic reticulum (SR), while activation of α_2-adrenoceptor (α_2) may promote contraction of smooth muscles mainly by increasing Ca^{2+} influx via opening of L-type voltage gated Ca^{2+} channels (L). G, GTP-binding protein; PLC, phospholipase C; PIP, phosphatidylinositol; DG, 1,2-diacylglycerol; C, Ca^{2+}-gated Cl^- channel.

Spontaneous contractile activity is observed in CSM strips [7] and isolated CSM cells [10]. This type of contractile activity is non-neurogenic and is not mediated by stimulation of autonomic receptors, but probably caused by spontaneous depolarizations secondary to mechanical stretch. Levin et al. [11] stressed that spontaneous contractile activity has no physiological significance in normal bladder function. However, its contributions to physiological function of the CSM and CC-EMG have not been elucidated.

The source of electrical potentials

Available evidence indicates that the CSM may not possess the capacity for regenerative electrical events (i.e., action potential) [6, 7]. Instead, electrical potentials are passively spread by means of intercellular diffusion of second messenger molecules and current-carrying ions through gap junctions [6]. It was demonstrated that the influx of Ca^{2+} through L-type VGCCs mediates a great part of the contractile activity of the CSM [10].

During the opening and closing of L-type VGCCs the time course of the membrane potential shows so called calcium spikes, which can be recorded extracellularly [10]. In vitro, the electrical activity of rabbit corpus cavernosum strip can be abolished by blockage of L-type VGCCs with nifedipine [10]. Probably, CC-EMG mainly reflects the summation of these synchronous electrical activities of a large group of the CSM cells.

The characteristics of CC-EMG

Both recording and interpretation of CC-EMG are difficult due to several factors. Firstly, CC-EMG is an extracellularly recorded signal, i.e., it reflects the effects of current flow in the extracellular space. Thus, even a small, concentric needle electrode will pick up the contributions of a large number of cells. Only the coordinated, synchronous action of a sizable number of cells can visibly influence the EMG, as a single smooth muscle cell is too small and its current-generating capability is too weak to make an individually recognizable impression on the signal as a whole. Further, the penis is a mobile organ, and CC-EMG is highly susceptible to movement artifacts and noise that are in the same frequency bands as CC-EMG. In addition, the information density of CC-EMG is low compared to that of the electrocardiogram (ECG), the electroencephalogram (EEG), and the striated muscle EMG.

Materials and Methods for CC-EMG Recording

Equipments and Settings

So far several systems have been used to record CC-EMG, such as Dantec Neuromatic 2000C (Dantec, Denmark), Space Recorder 7500 (Wiest, Germany), Dantec 15C01 EMG amplifier (Dantec, Denmark), and other nonspecific electrophysiological devices [12].

It became clear that the cutoff frequency of filters is crucial for the quality of the recorded signals. Stief et al. [4] demonstrated that narrowing of the frequency range from 0.1—20 Hz to 1—20 Hz resulted in a significant loss of amplitude of the signals, whereas narrowing to 5—20 Hz resulted in an almost complete loss of potentials (fig. 2-a). By applying a fast Fourier transformation (FFT), the frequency content of the signal can be studied [13]. Figure 2-b shows a plot of a FFT calculated from a CC-EMG recording. The

power of the signal is plotted as a function of the frequencies present in the signal. The figure clearly demonstrates that the most significant part of the power content is in the lower frequency range. Thereby by using high pass filters with improper cutoff frequency, the CC-EMG recording will be disturbed. The main question still to be answered is which cutoff frequency should be used to be still able to extract significant clinical information from the signal. The available cutoff frequency ranges in the literature are shown in table 1, and it seems that almost all the recordings lost a significant part of signal power. Furthermore, and this has never been recognized in CC-EMG literature before, the basic characteristics of filters can also influence the recorded signals. For example, by using filters with the same cutoff frequency but different filter order, the shape of the signals will be different (fig. 3). This implies that the results of different centers that use the same cutoff frequency but use different filter characteristics are also difficult to compare.

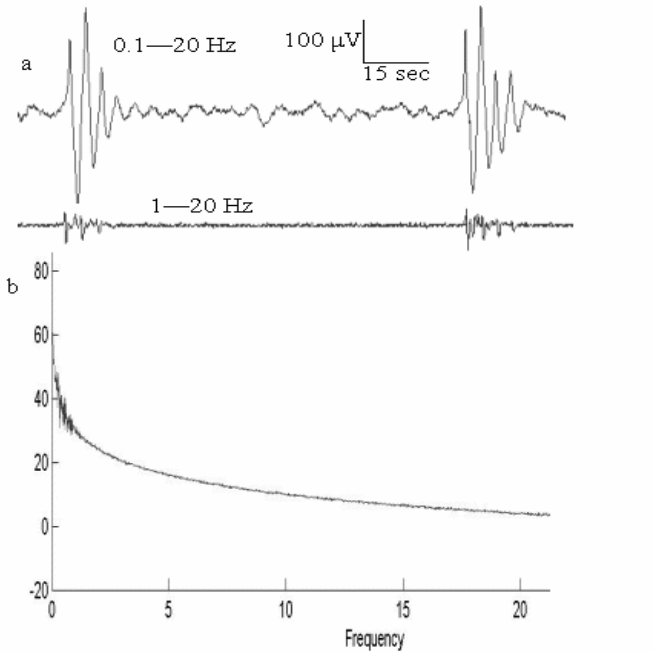

Figure 2 (a) Changing the cutoff frequency range of the filter in a 30 years old normal man resulting in significant differences of potentials. (b) Power spectrum of the raw signal without filtering of the same subject shows that the most significant part of the power content is located in the very low frequency range.

Table 1 Cutoff frequency ranges in the literature

Authors and time	Cutoff requency range (Hz)
Wagner, 1989 [1]	2--100
Stief, 1993 [18]	2--2000 and 0.5--100
Merckx, 1993 [2]	0.3--32
Sasso, 1996 [3]	0.5--32
Fabra, 1997 [5]	0.3--15
Basar, 1998 [20]	0.5--5000

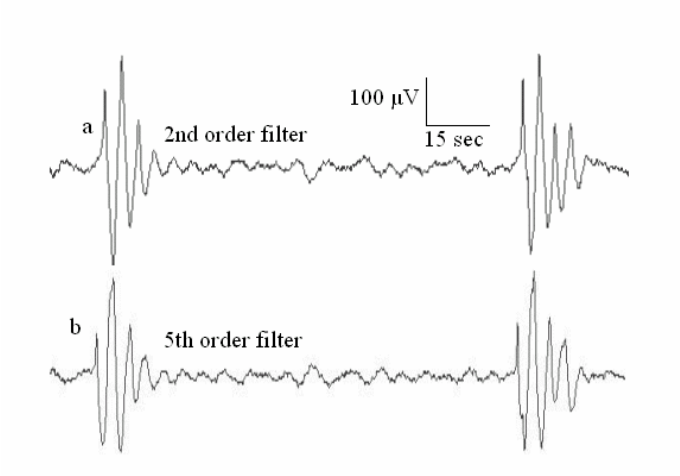

Figure 3 The same signal as fig.2, filtered with: (a) 0.1—20 Hz, 2nd order filters. (b) 0.1—20 Hz, 5th order filter. The shape of the signals is different.

Recently two new CC-EMG systems have been developed by Best Medical International (The Netherlands) and Medtronic (Denmark), respectively, which allow measurements with lower cutoff frequency of as low as 0 Hz. Both are portable, allowing uninterrupted for example overnight recording of CC-EMG at home.

Electrodes and Placement

Three types of electrodes have been employed: monopolar needle electrode, bipolar coaxial needle electrode, and surface electrode (Ag/AgCl) [2-5,12]. Stief et al. [4] and other investigators [2] described that the signals measured by surface electrodes are comparable with needle electrodes. Compared with needle electrodes, surface electrodes have several advantages:

1. Complete noninvasiveness.
2. Diminishing the abnormal recordings caused by higher adrenergic tone secondary to insertion of needle electrodes.
3. Picking up the electrical activity of a wider tissue area, allowing evaluation of the state of entire cavernous bodies more accurately [2,4].

The main disadvantage of surface electrodes is that they may record sympathetic skin response (SSR). However, usually SSR is provoked by different stimulus, is monophasic and with shorter duration, so it is not difficult to be distinguished from CC-EMG [14]. It may be concluded that surface electrodes have gained most popularity.

CC-EMG can be measured monopolarly or bipolarly. The former method uses one active electrode on/in the cavernous body and one reference electrode which is not located on the penis. The latter method measures potential difference between 2 monopolar electrodes (or between the 2 poles of a bipolar electrode) on/in the cavernous bodies. Even though almost no author described explicitly which method was used, according to the descriptions of the placements of the electrodes, it seems that most authors performed bipolar recording [2-5]. Based on the literature and taking into account that CC-EMG was recorded bipolarly, and that the potential difference between the electrodes was not zero, we conclude that the electrical activity within the corpora cavernosa is not completely synchronous, and some time delay must exist. Our preliminary results clearly show the time delay of electrical activities between 2 positions of the corpora cavernosa (Fig. 4). Thereby it is reasonable to suppose that the signals recorded bipolarly are strongly influenced by the exact locations of the 2 electrodes. For this reason, the comparison of different bipolar recordings with different locations of the electrodes is problematic. Obviously the comparison of bipolar recordings with monopolar recordings would be even more questionable. It is too early to judge which method is superior or if they can provide complementary information.

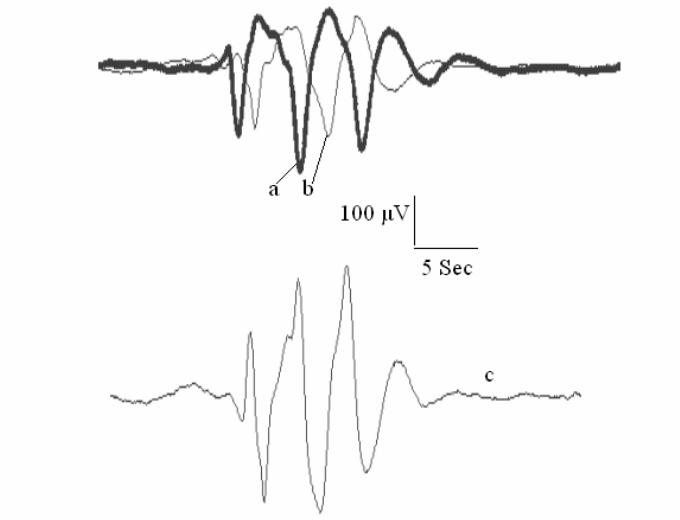

Figure 4 The time delay of electrical activities between 2 different positions of corpus cavernosum, and the formation of a bipolar signal. (a) a potential recorded with a electrode placed at the base of the right corpus cavernosum. (b) a potential recorded with a electrode placed 2 cm distally. (c) the bipolar signal, i.e. the potential difference between a and b (c=b-a).

Recording Conditions

The recording should be performed in a quiet room with a relaxed atmosphere, with the subject in a supine or sitting position. The subject is advised to relax during the examination, since stress may cause false result. However, at times over-relaxation leads to insufficient information content of the recording [12,15]. In most subjects, anarchic signals are found in the beginning, both with needle and surface electrodes, probably due to high adrenergic tone secondary to stress [2,3]. Therefore, registration should start after 10—30 min following placement of electrodes until the recording has stabilized. To obtain enough information, recording should last for at least 30 minutes [12]. The subject should not move during the measurement, in order to avoid movement artifact, and the examiner should be present to note artifacts immediately if they emerge.

Evaluation Methods

Firstly, the global recording should be evaluated: the quality of the recording (e.g. the occurrence of noise and artifacts, etc.), the baseline characteristics, the waveform of the potentials, and whether synchronous signal patterns are present (if multichannel

recording has been performed), could be gained [12]. Upon this, it is to be decided whether the recording is valid.

Then, the individual patterns of each potential should be analyzed minutely. It was recommended that not every potential but only the representative ones should be included to make the result more reliable. The evaluation parameters are:

1. Frequency: number of potentials per time unit.
2. Duration of the potentials.
3. Polyphasity: can be counted as the times of the transits through the baseline.
4. Amplitude: from the positive peak to the negative peak [12].

Wagner et al. [1] and other researchers [2,4,12] observed the changes of the potentials during the process of erection induced by audiovisual sexual stimulation (AVSS) or intracavernous injection (ICI) of vasoactive agents, and suggested that useful diagnostic information could be obtained by this method. However, this methodology has several disadvantages. Firstly, the subject's response to AVSS may be influenced by psychological factors, for example nervousness, embarrassment or lack of interest in the AVSS. Moreover, ICI is not able to give any information about the autonomic innervation. Measurement of CC-EMG during sleep may circumvent these problems.

To improve accuracy and reproducibility as well as to shorten the process of interpretation, Stief et al. suggested to digitize the CC-EMG data, and transform the potentials from the time domain to frequency domain (power density spectra) by FFT [13]. Later, the same group introduced 2 objective, time saving and easy-to-use programs, called discriminant analysis and artificial neural networks, to support the doctor to make his diagnostic decision without the need for a deep knowledge of the complex raw signal [16].

Recordings in normal subjects

In the literature there is much debate on what constitutes a normal CC-EMG recording. The recorded signals from different centers only showed some rough similarity but were not sufficient to perform a quantitative comparison. As described before this is at least partly due to the lack of standardization of the measurement and interpretation. Some investigators claimed that the potentials they recorded in normal potent subjects are reproducible intra-individually and comparable inter-individually, however, the variations of most parameters is so wide that it is difficult to define normative data [12,17]. Moreover, Fabra et al. [5], who performed 2 independent recordings in 36 healthy volunteers and 1 recording in 324 impotent patients, concluded that CC-EMG is not reproducible, and has limited value for differentiating potent subjects from those with ED. This conclusion does not come as a surprise, because on closer examination the prerequisites of standardization of equipments and methods have never been met.

Despite these deficits, some characteristics of CC-EMG in normal subjects can be defined. With the lower cutoff frequency of 0.5 Hz, most recordings show a flat baseline, or it is interrupted by a slow and continuous wave-like activity of not more than 75μV in amplitude at a rate of 4-8 times/min. The baseline activity is interrupted by a much stronger electrical activity, a so-called potential, in an irregular way, influenced by the degree of relaxation of the subject: the more relaxed the subject, the fewer potentials are seen, indicating stress dependency. The maximum peak to peak amplitude varies between 75 and >500 μV (needle electrodes). The duration of the potential, which is polyphasic, varies around a mean of 12s [12]. Synchronization of the potentials is revealed in studies in which the signals are recorded at 2 positions of the cavernous bodies with multichannel devices [1,17]. Dramatic changes of potentials are observed during tumescence and erection induced by AVSS: with increasing tumescence and rigidity, an increase of frequency of the potentials with a simultaneous decrease of amplitude and polyphasity is seen [4,12,17]. During full erection, potentials with low amplitude but high frequency can still be recorded [4,17]. In contrast, no potential (electrical silence) is recorded during full erection following ICI [2,17]. It is noteworthy that some normal subjects showed "abnormal" recordings: either almost no potential or anarchic, desychronous signals were observed [12]. It can be concluded that for the future development of CC-EMG,

clarification of potentially disturbing factors other than equipment and electrode problems or movement artifact is essential.

Preliminary Observations in Patients with ED

It is without doubt that any conclusion about the diagnostic importance of CC-EMG in patients with ED is questionable, before the aforementioned problems have been solved. However, clinical studies have turned out some promising findings.

Stief et al. postulated that short lasting potentials with reduced amplitude, and "whips" (short potentials with a fast convex phase of depolarization and a slower repolarization phase of concave shape) indicate peripheral autonomic lesions, whereas potentials of low amplitude, irregular shape and slow depolarization are suggestive of CSM degeneration [17,18]. To verify this assumption, Sattar et al. [19] correlated the CSM content in penile biopsies of impotent patients with their CC-EMG and concluded that CC-EMG has a sensitivity of 60% and a high specificity (100%) in predicting abnormal low CSM content. In contrast, Basar et al. [20] did not observe an ultra-structural difference between tissue sample from diabetic patients with a low amplitude CC-EMG and non-diabetic patients with a high amplitude CC-EMG. No change or even an increase of electrical activity following AVSS or ICI is supposed to indicate CSM degeneration or a discoordination within the autonomic nervous system [1,21].

In reviewing the literature, the following observations have been described in patients with ED:

1. Diabetes mellitus: Irregular potentials with low amplitudes and slow depolarization are obtained in the flaccid state, which do not change following ICI [17,18,20]. Some authors observed a trace characterized by very long intercomplex intervals or no activity over periods of 30 min [2].

2. Peripheral autonomic nerve impairment caused by pelvic operation or trauma: Stief et al. [17,18] observed potentials of low amplitude, high frequency, irregular

periodicity, and asynchronism. In addition, “whips” were seen. Some patients showed abnormal as well as normal potentials, especially patients after nerve-sparing radical retropubic prostatectomy, suggestive of partially disrupted peripheral autonomic supply. Sasso et al. [3] demonstrated that following radical cystectomy, the impotent patients showed a typical pattern of low-amplitude/low-frequency potentials, whereas after nerve-sparing cystectomy, all of the impotent patients showed a mean amplitudes similar to controls. They suggested that CC-EMG seemed able to differentiate neurological from arterial lesions, leading to a correct therapeutic choice.

3. Spinal cord lesion: None of the CC-EMG recordings were entirely normal; however, abnormal potentials and “whips” as well as rather normal looking potentials were observed. Often potentials with a longer duration than normal were seen. In most patients with a high complete spinal cord lesion (above the level of Th1) no normal potentials with synchronization of the cavernous bodies were found. Often, potentials of high frequency and irregular shape were seen as a permanent electrical activity without a silent baseline [18].

4. Veno-occlusive dysfunction (VOD): VOD may be functional or organic. The former is caused by the insufficient relaxation of the CSM to compress the venules against the tunica albuginea rather than a localized defect in the sub-tunical or intra-tunical venous compression system. Theoretically this type of VOD will not benefit from penile venous surgery. CC-EMG was supposed to be able to distinguish functional VOD caused by CSM degeneration from organic VOD, playing an important role in therapeutic decision-making [22].

Current Difficulties and Future Prospect

Over the past several years few new results of CC-EMG studies have been published, reflecting the difficulties most clinical investigators experience in solving the fundamental and technical questions that come on their way. We expect that the co-operation between several research groups, including physiologists, neurologists and

engineers, as facilitated by the European Commission with in the COST Action (B18), will provide sufficient critical mass to revitalize the application of CC-EMG as a clinical tool, not only to diagnose ED but also in a broader context to diagnose the vascular system.

Having defined the key-difficulties at present, future study targets have been defined as followings:

1. Increasing our understanding of the ultra-structure and electrophysiology of corpus cavernosum.

Obviously, this fundamental knowledge is essential to understand the origin of electrical signals, the aspects of normal CC-EMG, and the clinical meaning of abnormal signals. The fundamental problems to be solved have been mentioned above.

Since the introduction of CC-EMG, doubts whether the recorded signals are actually the electrical activity of the CSM have never stopped. The signals showed pronounced differences during flaccidity and after erection in human [1,3,4,12], before and after denervation in animals [23], indicating that CC-EMG is from the cavernous bodies, not caused by distant electrical activities. The signals recorded from rat urethra surface significantly decreased following stimulation of the pelvic nerve, but were not altered by temporary closure of aorta [24], ruling out the possibility that the signals were caused by changes in volume of the corpus cavernosum. Colakoglu et al. [15] assumed that the signals recorded from corpus cavenosum were caused by the summed contractions of the CSM rather than the electrical activities. However, Vardi et al. demonstrated that erection induce mechanically by introcavernous injection of saline did not influence the cavernous electrical activity [25], while the retractile movement of the penis might be abolished or at least diminished during erection. On the other hand, the possibility that CC-EMG is partly influenced by the contractile movements of the CSM cannot be ruled out [11].

2. Solve technical problems

The main tasks include:

2.1 Define the proper recording method (monopolar, bipolar, or combine of these 2 methods), and the exact locations of the electrodes.

2.2 Optimal the filter and the cutoff frequency range.

2.3 Avoid or discriminate artifacts and noise from the bio-signals.

2.4 Establish an easy and accurate method to interpret the recorded signals.

3. Define normative data and diagnosis criteria

As mentioned above, the crucial problem for the clinical study is that to date no normative data has been established, which makes differentiation between "normal" and "abnormal" recordings questionable.

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Chapter 3

The Methodology of Corpus Cavernosum Electromyography Revisited

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Abstract

Objective: The methodology of corpus cavernosum electromyography (CC-EMG) was revisited, in order to overcome current methodological difficulties that hinder its clinical application.

Materials and methods: Using an 8-channel device, CC-EMG was performed in 12 healthy volunteers. Surface electrodes were placed bilaterally on the penile shaft and the kneecap (reference electrode), the pubis region and the anterior superior iliac spine (ASIS). A band pass filter with cut-off frequencies of 0.1 and 20 Hz was used. At least 2 sessions of recordings were performed in each subject.

Results: Thirty-five of 46 recordings were interpretable. Significant time delays between potentials recorded from different sites of the CC were detected. Clear spatial voltage gradients related to CC-potentials were observed on the pubis region. No voltage changes related to CC-potentials, but electrical activity from other sources were recorded from the ASIS. In contrast to frequency, a clear correlation could be demonstrated between amplitude, duration and polyphasity of CC-potentials recorded in 2 different sessions in the same individual.

Conclusions: Multichannel monopolar recording of CC-EMG with surface electrodes is practical and has several advantages compared with bipolar recording. The results provide evidence that the recorded signals indeed reflect electrical activity of the CC and therefore offer a basis to pursue further clinical validation studies.

Keywords: Penis; Corpus cavernosum; Electromyography; CC-EMG; Monopolar

Introduction

In 1989 corpus cavernosum electromyography (CC-EMG) was introduced by Wagner and co-workers as a promising new method to evaluate the function of cavernous smooth muscle and its autonomic innervation [1]. Although several research groups have worked intensively on its clinical development [2-5], CC-EMG has failed to mature into a useful clinical tool. The most important reason for the lack of progress in this field is that doubts whether the signals indeed reflect electrical activity of the corpus cavernosum (CC) persist. Moreover, a lack of standardization of equipment, measurement and interpretation protocols made that the recordings of different centers could not be used for a quantitative comparison of patients with similar disease entities. Within the framework of the EU COST Action B18 program "corpus cavernosum EMG in erectile dysfunction" in which 22 scientists from 12 counties participate, we revisited the methodology of CC-EMG. In this study, the revised methodology is validated in a population of healthy volunteers.

Materials and methods

Equipment

An eight-channel Portilab Screener system (TMS International, Enschede, The Netherlands), connected to a portable computer (Toshiba Satellitepro6100), was used to record CC-potentials. The electrodes used were pre-gelled surface electrodes Medtronic 9021S0231 (1.5 * 2.0 cm in size, Medtronic, Copenhagen, Denmark).

Study population

Measurements were performed in 12 healthy volunteers with a mean age of 25.5 years (range 19-31). Body mass index (18-25 kg / m^2), blood pressure (systolic 90-150 mmHg, diastolic 60-90 mmHg) and pulse rate (50-120 beats/min) were in normal range. Within 12 hours prior to the measurements, alcohol, coffee, smoking and sexual activity was not allowed. Informed consent was obtained from each subject, and at least 2 recordings were performed with an interval of at least 24 hours.

Study protocol

Measurements were performed between 8 and 12 am, in a closed, semi-dark room with the examiner present. The room temperature was between 20 and 25 degree. The subject was placed in the supine or 45-degree sitting position on an examination table and was asked to relax as much as possible. The areas where the electrodes were to be placed were shaved without injury of the skin. Before application of the electrodes the skin was abraded lightly using a gauze and Nuprep gel (Weaver & Co., Nucla Way, USA) for about 10 strokes, and then was cleaned with alcohol to remove scales and to improve electrode adhesion. Depending on the size of the penis, four or six surface electrodes were placed on the shaft bilaterally (2 at the base of the penis, 2 close to the coronal sulcus, and 2 in between, if possible). After several pilot measurements (see discussion), it was decided to place the reference electrode over one of the kneecaps. The grounding electrode was placed on the thigh of the same side as the reference electrode. In 6 subjects, 4 additional electrodes were placed in the midline of the pubis and the anterior superior iliac spine (ASIS), aiming to observe electrical activity from these regions. Recording began after 10-20 minutes equilibration, lasting at least 20 minutes during flaccidity. Unfiltered as well as filtered signals were recorded simultaneously and stored digitally. The sampling frequency was 128 Hz. Filters with different cut-off frequencies were tested using the Matlab software (the MathWorks, Inc., Natick, Mass., USA), and eventually a band pass filter with cut-off frequencies of 0.1 and 20 Hz was used.

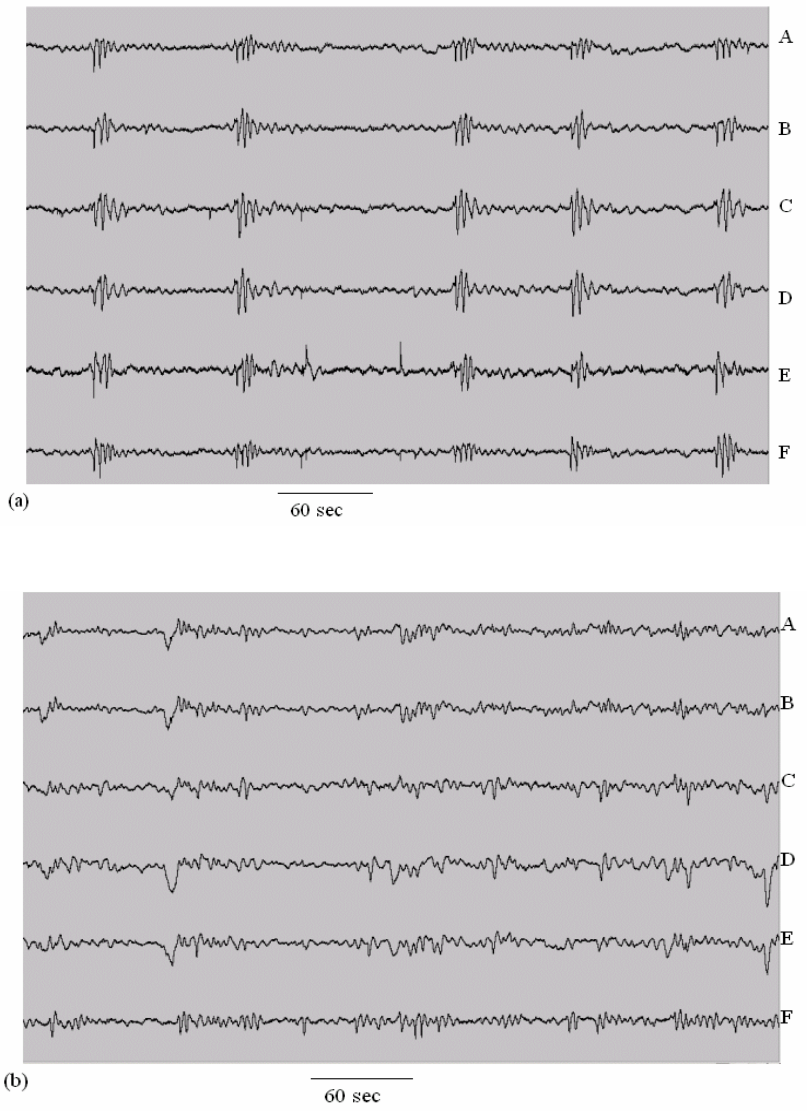

Figure 1. (a) An interpretable recording from a 25 yr old healthy man. (b) A "non-interpretable" recording from a 24 yr old healthy man. A, B and C are from the left cavernous body, from the proximal to distal part in order; D, E and F are from the right side, from distal to proximal part in order.

Evaluation and analysis of the recordings

Firstly, the recordings were evaluated globally. Attention was paid to the quality of the recording (e.g. the occurrence of noise and artefacts), the baseline characteristics, and the waveform of CC-potentials. The recordings with a stable baseline, and reproducible CC-potentials which were distinguishable from the baseline were regarded as good quality recordings. Upon this, whether the recordings were interpretable or not could be decided

(Figure 1a and 1b show examples of interpretable and "non-interpretable" recordings, respectively). Secondly, the temporal relationship of CC-potentials recorded from different sites of the CC, and the relationship of signals from the CC, the pubis and the ASIS were examined. Finally, the individual CC-potentials were analysed and the intra-individual reproducibility was determined, on basis of the first 2 interpretable recordings of each subject. Recordings from the 2 electrodes at the base of the penis were used and the parameters amplitude, polyphasity, duration and frequency (number of potentials per 10 minutes) were determined. For the first 3 parameters, the 10 most representative CC-potentials with definite beginnings and endings (5 from each side) were selected and analysed. Pearson correlation analysis was used, and a p-value < 0.05 was considered statistical significant.

Results

CC-potentials were strongly influenced by the cut-off frequencies of filters. Changing the lower cut-off frequency from 0.1 to 1 Hz resulted in a significant loss of the amplitude of CC-potentials. Changing the lower cut-off frequency from 0.1 to 0.05 Hz did not influence the amplitude of CC-potentials significantly, but the baseline became less stable (Fig 2). Hence most of the signal power appeared to be between 0.1—1 Hz, and a band pass filter with cut-off frequencies of 0.1 and 20 Hz was considered to be the proper set-up.

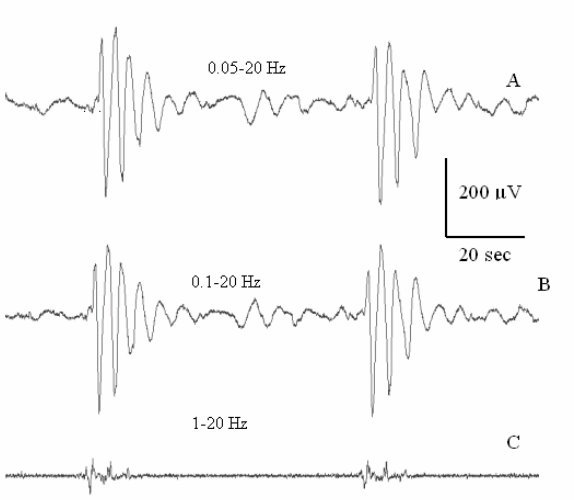

Figure 2. A, B and C demonstrate the influence of changing lower cut-off frequency on CC-potentials. Most of the signal power seems to be between 0.1 to 1 Hz.

Thirty-five of 46 recordings (76%) showed a stable baseline and interpretable CC-potentials, which could be easily analysed. Eleven recordings showed an unstable baseline, continuous oscillations, or CC-potentials interfered by disturbing signals or noise, making them impossible to analyse. The quality of the recordings seemed to be individual-dependent: all 4 recordings from a 24 years old subject and 4 out of 6 recordings from a 30 years old subject were "non-interpretable", while the other 10 subjects contributed 3 "non-interpretable" and 33 interpretable recordings. No clear physical, environmental, or psychological differences could be detected between those 2 and the other 10 subjects. See figure 1a and 1b.

For the interpretable recordings, usually irregular signals were observed in the beginning, and gradually the recordings became stable, regular and interpretable. The time for stabilization differed among the subjects as well as intra-individually, varying from 0 to 20 minutes. The baseline was flat or showed slow wave-like activity, interrupted by the typical polyphasic CC-potentials. CC-potentials emerged non-periodically and the quality of the recordings and the frequency of CC-potentials seemed to be stress-dependent: the more relaxed the subject was, the better quality of the recordings, and the fewer CC-potentials. Sometimes, when a subject dozed off, "electrical silence" without any CC-

potentials lasting for more than 10 minutes was observed. Often, after a period of "electrical silence", a group of CC-potentials or even a continuous long signal emerged.

CC-potentials recorded from different sites of the CC were not synchronous. Depending on the interelectrode distance, time delays were observed (around 0.5 second if the interelectrode distance was around 2.5 cm). In general, CC-potentials from proximal sites emerged earlier than those from distal sites. A few times, CC-potentials only existed at one of the cavernous bodies or even only from one electrode, while very weak oscillations were observed from other electrodes (Fig. 3).

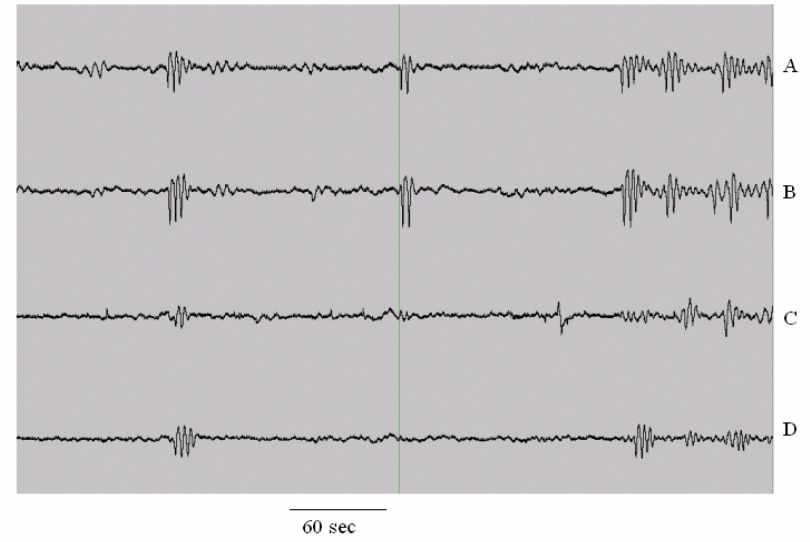

Figure 3. The vertical line indicates that CC-potentials are only picked up from the left cavernous body (channel A and B), while weak oscillations are observed from the right side (channel C and D). A and D are from the base of the penis, B and C are from the distal part of the penis.

The electrodes placed on the pubis region could also pick up electrical signals related to CC-potentials. However, their amplitude was lower, indicating a spatial voltage gradient: The further the electrodes were away from the CC, the lower the amplitudes of CC-potentials observed (Fig. 4). From the ASIS, no activity related to CC-potentials, but significant electrical activity from the heart and other sources (bowel, bladder?) could be picked up (Fig. 5).

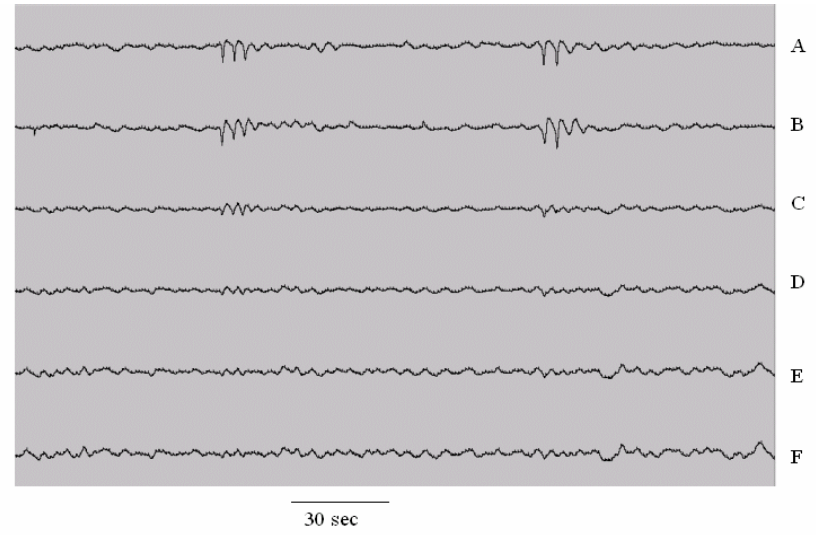

Figure 4. A and B are from the base of the penis, C-F are from the mid line of the pubis region, 1.3; 3.1; 5; 6.4 cm (from the middle of the electrodes) to the base of the penis in order. The width of the electrodes is 1.5cm. A clear spatial voltage gradient on the pubis region can be seen.

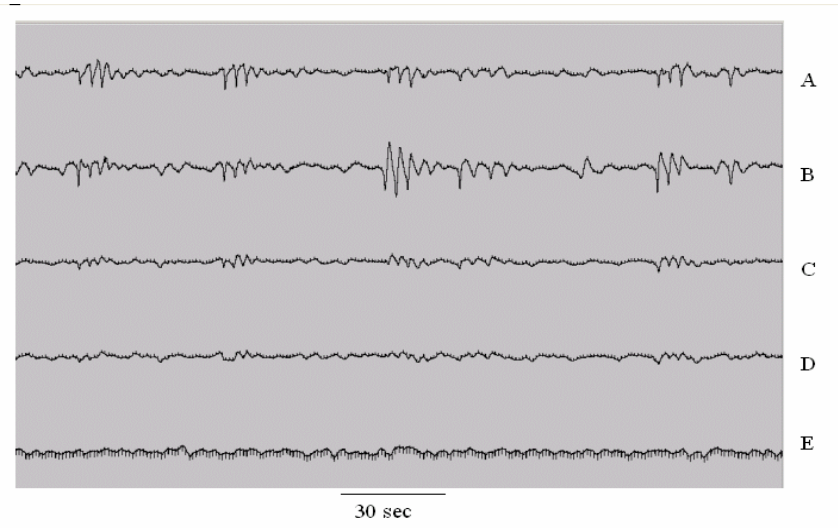

Figure 5. A and B are from the base of the penis, C and D are from the mid line of the pubis region, 1.3; 3.1 cm to the base of the penis in order. E is from the right ASIS. A clear spatial voltage gradient on the pubis region can be seen. No voltage changes related to CC-potentials, but significant EA from the heart (ECG) is recorded from the ASIS (E).

Table 1 lists the parameters of CC-potentials and correlations between 2 recordings in 10 normal volunteers. The subject who contributed 4 “non-interpretable” recordings was not included. In the second measurement of another subject, the recording from the left side

of the base of the penis showed much noise which severely disturbed CC-potentials, and therefore, this subject was also excluded from the correlation analysis. The amplitude, duration and polyphasity of CC-potentials in the 2 recordings showed a strong correlation, whereas the frequency of CC-potentials did not.

Table 1 Parameters of CC-EMG potentials and correlation of 2 recordings in 10 healthy volunteers (data are presented as the mean +/- SD)

	Recording 1	Recording 2	r	p-value
Amplitude (μV)	381.3 ± 79.1	365.6 ± 83.9	0.78	0.008*
Duration (sec)	13.26 ± 3.61	13.76 ± 3.21	0.94	0.000*
Polyphasity	6.57 ± 2.07	6.91 ± 1.55	0.85	0.002*
Potentials/10min	9.97 ± 6.01	9.28 ± 4.22	0.60	0.070

* Significant

Discussion

So far, several systems have been used to record CC-potentials [6, 7]. To our knowledge, only two studies reported multichannel (>2 channels) recordings [8, 9]. The authors applied this method in a small group of patients, for a purpose of observing the relationship between signals from the CC and the limbs, and actually no multi-channel recordings from different sites of the CC were shown. In the literature, most studies used one or two channel devices, making the assessment of temporal relationship between potentials from different sites of the CC and investigation of spatial voltage gradients between adjacent regions impossible. However, both the assessment and the investigation are important for determining the origin of recorded signals. In this respect, the device used in this study had several advantages. Firstly, the availability of eight channels facilitated the study of the temporal relationship between CC-potentials and spatial voltage gradients. Secondly, monopolar as well as bipolar signals could be recorded, and the existing software made the post-hoc simulation of bipolar recordings out of two monopolar signals possible. Finally, the adjustability of the filter cut-off frequencies made the assessment of the optimal cut-off frequencies possible.

Monopolar recording facilitates the assessment of a potential difference between the electrode placed directly over/in the bioelectric source of interest and the reference

electrode at a distant site at which there is no signal from this source present. Bipolar recording means that the potential difference is recorded between two electrodes (or two poles of a bipolar electrode) placed over/in the bioelectric source [7, 10]. Until now, most CC-EMG studies are based on bipolar recordings [1-5]. Since a bipolar signal is a deduction of potentials between 2 monopolar electrodes, a substantial part of information may be lost. Furthermore, taking the significance of time delays of CC-potentials from different sites of the CC into account, one can imagine that the signals recorded bipolarly are dependent on the exact location of the electrodes. Placing the electrodes bilaterally or unilaterally, 2 cm or 5 cm away will make a significant difference. Moreover, because the dimensions of the penis vary widely depending on penile tumescence, it is difficult to fix the interelectrode distance exactly. Therefore, it would be problematic to make an intra-individual, inter-individual or inter-institutional comparison of bipolar recordings. These problems do not exist in monopolar recording. Although bipolar recording has the advantage that it may reduce unwanted signal components [10], all the information contained in bipolar recording is present in monopolar recording, since a post-hoc bipolar montage scheme can be derived from monopolar raw data, but not vice versa [10]. Therefore, we used monopolar recording instead of the traditional bipolar recording to revalidate the methodology of CC-EMG and to make the methodology suitable for multicenter application.

In monopolar recording, the ideal position of the reference electrode is at the site where the electrical field induced by the bioelectrical source has declined to zero; Furthermore, at this position, no specific electrical activity should be generated by itself (i.e. electrically inactive region) [10, 11]. At first the pubis was under consideration, however, clear voltage changes related to CC-potentials were recorded at this location. The ASIS was then chosen. However, electrical activity of the heart and other sources (bowel, bladder?) were picked up from the ASIS, and significantly affected CC-potentials. Due to the following characteristics, the kneecap turned out to be the optimal reference position: (1) It is far away from the CC and other organs containing smooth muscle. (2) There are no major muscles or nerves underlying the skin. With this placement, most of the recordings showed a stable baseline and interpretable CC-potentials.

Despite efforts to rule out confounding factors (a strict study protocol was used, involving only young healthy volunteers who were prohibited to drink alcohol and coffee, smoke or have sexual activity 12 hours prior to the measurements, the room temperature was kept constant and all the measurements were performed by the same investigator [X.G.J.]), one in four recordings was "non-interpretable". In the literature, "non-interpretable" recordings are reported in about one in three subjects [6]. This may be partly due to improper skin preparation, electrodes placement or faulty connections, stress in the subject or movement artefacts. Additionally, tumescence changes due to skin preparation and electrode placement may play a role in inducing sympathetic activity and thus inducing irregular signals. Also, the electrode gel needs time to penetrate the skin to build a stable gel-skin interface [12]. However, the fact that the quality of the recordings in the present study was individual-dependent cannot be explained by these factors. We speculate that the reproducible "non-interpretable" recordings are caused by an intrinsic high basic sympathetic tone of the subject. The consistent finding in the literature and the present study of irregular signals in the beginning of the measurements which is explained by a high sympathetic tone due to "stress" or anxiety supports our speculation [2, 4]. Further studies in more subjects are needed to verify this hypothesis and to clarify the underlying mechanism.

As described earlier [7], the optimal filter cut-off frequencies are still to be decided. Stief et al. demonstrated that the signals are in a frequency range below 5 Hz [4, 13, 14]. In accordance with their observation, our results clearly show that most of the signal power is between 0.1—1 Hz: Decreasing the lower cut-off frequency below 0.1 (to 0.05 Hz) does not influence the amplitude of the CC-potential significantly, whereas the included low frequency baseline fluctuations may impair the interpretation of CC-potentials. Therefore, 0.1 Hz is recommended as the lower cut-off frequency. The higher cut-off frequency should be at least above 5 Hz, but below 50 Hz to avoid the 50 Hz mains noise. Hence, we recommend 20 Hz as the higher cut-off frequency.

Since the introduction of CC-EMG, doubts whether the recorded signals reflect electrical activity of the CC have persisted. Other possibilities suggested in the literature include distant electrical events [8], retractile movements of the penis [15], changes in volume of the penis caused by blood flow [16], and sympathetic skin response (SSR) [8]. The results of this study provide strong evidence that the recorded signals indeed reflect electrical activity of the CC: Firstly, the non-synchronicity of potentials recorded at different sites of the penile shaft proves that they are generated from the penis. It is a common knowledge that signals from a distant source would be synchronous [17]. Furthermore, the existence of spatial voltage gradients related to CC-potentials measured on the pubis region indicates that the penis, rather than other organs, is the source of the recorded signals. Moreover, the existence of signals on the pubis region accompanying CC-potentials rules out that CC-potentials are caused by the retractile movements or changes in volume of the penis, since it is unlikely that the retractile movements or changes in volume also occurs there. Finally, the facts that the signals recorded at the pubis were not identical to signals recorded at the penis but appeared to be spatial voltage gradients, and their absence at a more distant region (the ASIS) indicates that they are not SSR, because SSR is a widespread and not a local phenomenon.

Besides so-called representative CC-potentials, a substantial number of "non-representative" CC-potentials were encountered, for example CC-potentials disturbed by movement artifacts or noise, potentials with low amplitude and short duration (i.e. signals from a distant source), or continuous long signals. If all these potentials were included for quantitative analysis, the value of the parameters would have been variable and unreliable. Therefore, in line with the literature [6] only representative CC-potentials were included for the assessment of reproducibility. The results show that amplitude, duration and polyphasity of CC-potentials are reproducible, whereas frequency, i.e. number of CC-potentials per time unit is not. In the literature, only one study reported reproducibility analysis of 2 independent recordings [5]. Although the authors concluded that the parameters of CC-potentials are poorly reproducible, on closer examination of their data, parameters such as amplitude and duration of CC-potentials appeared to be reproducible, whereas polyphasity was not analyzed. The most irreproducible parameter

was number of potentials per time unit. The fact that the latter is not reproducible does not come as a surprise, since the number of CC-potentials per time unit is highly dependent on the sympathetic tone of the subject, which can vary significantly.

Conclusion

The objective of this study was to revalidate the methodology of CC-EMG, using state-of-the-art equipment and strict protocols, within the framework of a European collaboration. We could demonstrate that multichannel monopolar recording of CC-potentials has several advantages as compared to the traditional bipolar recording. Therefore, a multichannel equipment allowing to perform monopolar recording should be used. The filtering setting of the equipment should be adjustable, and a band pass filter with cut-off frequencies of 0.1 and 20 Hz is recommended. The parameters: amplitude, duration and polyphasity of CC-potentials appear to be reproducible, while frequency does not. By using this methodology time delays between CC-potentials recorded from different sites of the CC and spatial voltage gradients at the level of the pubis region could be demonstrated. These results provide strong evidence that the recorded signals indeed reflect electrical activity of the CC and therefore offer a basis to pursue further clinical validation studies.

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Chapter 4

Corpus Cavernosum Electromyography during Morning Naps in Healthy Volunteers: Further Evidence that CC-potentials Reflect Sympathetically Mediated Activity

Xiaogang Jiang, Eric J.H. Meuleman, Hessel Wijkstra, Gorm Wagner

J Urol 2005;174:1917-1920.

Abstract

Purpose: to assess the practicability of corpus cavernosum electromyography (CC-EMG) during morning naps in the laboratory, and to further validate this method.

Materials and methods: Eleven healthy volunteers with a mean age of 23.8 years (range 19-31) were included. CC-EMG measurements started between 6:30 to 7 o'clock in the morning. Two surface electrodes were placed at the base of the penis bilaterally, and a reference electrode was placed on one of the kneecaps. A strain gauge or Barlow gauge was used to monitor the changes of penile circumference. The subjects were asked to sleep. The recordings lasted for 2 to 3 hours. Two recordings were performed in each subject.

Results: Full erections were observed in 17 of the 22 recordings (77%), partial erections in 3 recordings (14%), and no tumescence in the other two recordings (9%). CC-potentials consistently disappeared during tumescence and erection, while continuous CC-potential oscillations reappeared during detumescence. During flaccidity both bursts of CC-potentials and "electrical silence" were recorded. Penile shrinkage was observed accompanying CC-potentials but not during "electrical silence".

Conclusions: CC-EMG during morning naps is a practical and valid method to investigate the electrophysiology of the corpus cavernosum. The patterns of CC-EMG signals during tumescence, detumescence and flaccidity fit in the existing theory that CC-potentials reflect sympathetic mediated activity of the cavernous smooth muscle.

Keywords: CC-EMG, corpus cavernosum, smooth muscle, sleep, electromyography

Introduction

Although already fifteen years have passed since corpus cavernosum electromyography (CC-EMG) has been introduced to study the electrophysiology of the human corpus cavernosum (CC) [1], the understanding of the recorded signals is far from complete. Therefore, the use of CC-EMG as a clinical diagnostic methodology still is controversial. Based on the notion that the recorded signals (CC-potentials) reflect sympathetically mediated contraction of the cavernous smooth muscle (CSM) [2, 3] one would expect that CC-potentials decrease or even cease during relaxation of the cavernous smooth muscle i.e. during penile tumescence and erection. However, instead of a decrease, an increase of CC-potentials has been described during erection [4-7]. These confusing measurements were interpreted as reflections of skin electrical activity, movement artefacts or external noise and has raised questions with regard to the validity of the method [6]. Finally, it has lead research groups to abandon the further development of CC-EMG into a clinically useful diagnostic tool.

Following a recent revalidation of the CC-EMG methodology [8] we set out to observe CC electrical activity during sleep in healthy volunteers. The hypothesis was that the "contradictory" observations in the literature may have been caused by the fact that all studies had been performed under unphysiological conditions, i.e. in audiovisually or pharmacologically evoked erections in the laboratory and in patients with a broad spectrum of erectile dysfunctions [1, 4-7].

Materials and methods

Study population

Eleven healthy volunteers with a mean age of 23.8 years (range 19-31) were included. Body mass index (18 - 25 kg / m^2), blood pressure (systolic 90--150 mmHg, diastolic 60--90 mmHg) and pulse rate (50—120 beats/min) were in a normal range. The volunteers were asked to refrain from alcohol, coffee, smoking and sexual activity within a period of 12 hours prior to the measurements. Informed consent was obtained from each subject.

Seven out of the 10 subjects had participated in CC-EMG studies before. A minimal fee was paid for participation.

Equipment

A Screener system (TMS International, Enschede, The Netherlands) connected to a portable computer (Toshiba Satellitepro6100) was used to record CC-EMG signals and a strain gauge was used to monitor the changes in penile volume in the first 8 subjects. For the last 3 subjects, a newly developed system Porti from the same company was used. The amplifier parts of the 2 systems were the same. One of the advantages of the Porti system was that it could be connected to a Barlow gauge (Behavioral Technology, Inc., Salt Lake city, U.S.A.) for simultaneous recording of the changes in penile volume. The electrodes used were pre-gelled surface electrodes Medtronic 9021S0231 (1.5 * 2.0 cm in size, Medtronic, Copenhagen, Denmark).

Study protocol

Measurements started between 6:30 to 7:30 am in a quiet and semi-dark room with the examiner present. The room temperature was between 20 and 25 degrees. The subjects were supine. Two surface electrodes were placed at the base of the penis bilaterally, a reference electrode was placed on one of the kneecaps, and a grounding electrode was placed on the thigh at the same side of the reference electrode. A strain gauge or Barlow gauge was placed around the mid shaft of the penis. A band-pass filter with cut-off frequencies of 0.1 and 20 Hz was used to process the recorded signals. The subjects were asked to close their eyes and try to sleep. The subjects' penis was uncovered to allow the examiner to observe subtle changes in penile volume. The measurements lasted for 2 to 3 hours. Because a sleep laboratory was not available for this study, the state of sleep was judged by the examiner and the subjects' self-report afterwards. In each subject, two recordings were performed with an interval of at least 24 hours. The investigator's visual observation that the penis was erect and a plateau on the strain gauge graph defined full erection. Any penile enlargements less than these were regarded as partial erection.

Results

The sleep quality and the erectile episodes during 22 recording sessions of the 11 subjects are shown in Table 1. In brief, full erections were observed in 17 of the 22 recordings (77%), partial erections in 3 recordings (14%), and no tumescence in the other 2 recordings (9%). During both visits of subject 3 and the second visit of subject 8 and 10, the subjects woke up when full erections occurred, and detumescence occurred instantly. During the rest of the episodes of penile erection the subjects were asleep. Both recordings of subject 5 showed severe disturbing signals, making the analysis of the recordings impossible. The only special situation of this subject was that he often had night duty and did not have a regular sleep habit. The two recordings of this subject were excluded from further analysis.

Table 1: Sleep quality and erectile episodes during 22 recording sessions of the 11 volunteers

	Full erection	Partial erection	No erection	Total
Good sleep	16	1	0	17
Poor sleep	1*	2	2	5
Total	17	3	2	22

*The full erection was induced when the examiner replaced the electrodes during semi-sleep state

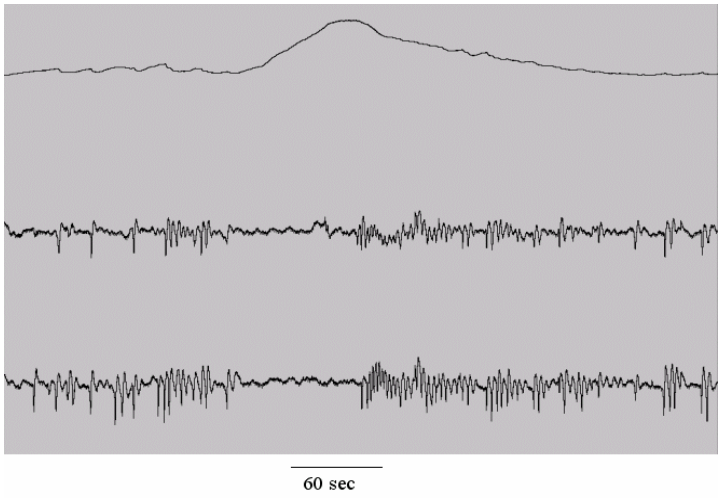

Figure 1. A part of a CC-EMG recording during a morning nap in a 21 years old healthy man (subject 10). A sleep related erection occurred. The upper tracing represents the changes in penile circumference measured by the Barlow gauge. The middle and lower tracing are CC-EMG recordings from the left and right sides of the base of the penis, respectively.

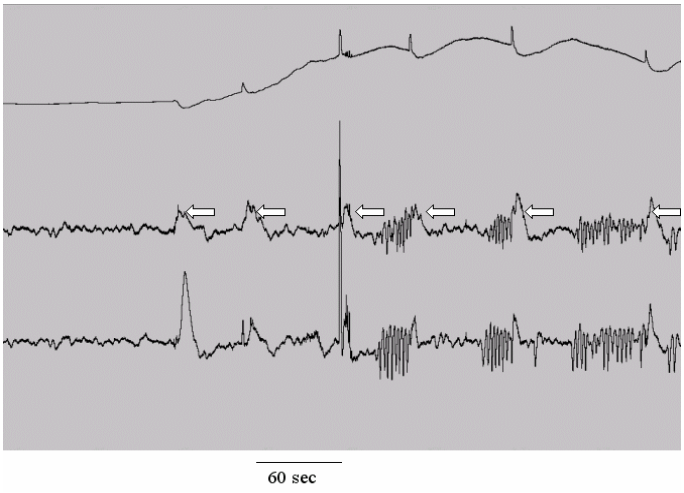

Figure 2. A part of a CC-EMG recording during a morning nap in a 22 years old healthy man (subject 11). A sleep related erection occurred. The set-up of the Barlow gauge and the electrodes are the same as Figure 1. The arrows indicate the movement artefacts associated with contractions of the ischiocavernosus and bulbocavernosus muscles.

CC-potentials consistently disappeared during tumescence and erection, while continuous CC-potential oscillations reappeared during detumescence lasting for several minutes, and then gradually returned to the basal level accompanying the penis reaching flaccidity

(Fig. 1). Movement artefacts associated with the change in position of the penis or the contractions of the ischiocavernosus and bulbocavernosus muscles were seen during tumescence, erection and detumescence in the most of the recordings (Fig. 2). These movement artefacts could be easily distinguished from CC-potentials, since they had clearly a different waveform. Instead of the common pattern of tumescence followed by full erection (or partial erection) and then detumescence until reaching flaccidity, sometimes the penis fluctuated between tumescence and detumescence for several times before reaching complete flaccidity. CC-potentials were always absent during increases of penile circumference, while continuous oscillations were always associated with decreases of penile circumference (Fig. 2).

During flaccid state, 11 recordings showed a pattern where the CC-potentials occurred in bursts, i.e. a group of CC-potentials followed by a period of "electrical silence" (Fig. 3). In the other 9 recordings, CC-potentials did not appear in bursts, but still a period of "electrical silence" could be observed. The duration of "electrical silence" could be longer than 10 minutes. Synchronously with the occurrence of a CC-potential, a short episode of shrinkage of the penis was observed. This phenomenon was reflected by the strain/Barlow gauge measurements as changes of penile circumference (Fig. 4). During "electrical silence", the volume of the penis gradually increased.

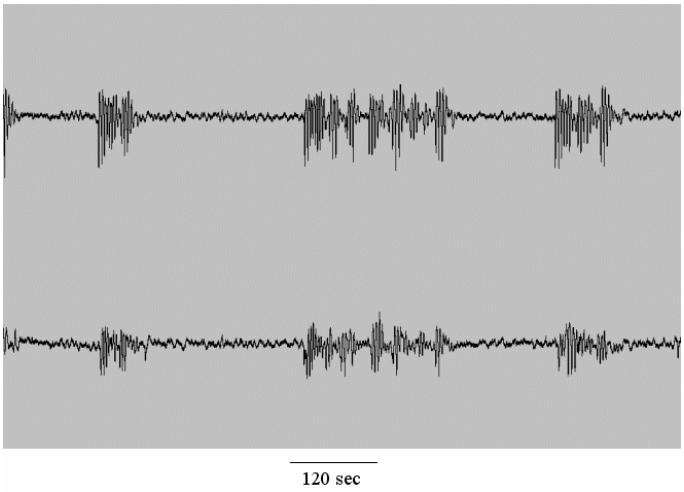

Figure 3. A part of a CC-EMG recording during a morning nap in a 22 year old healthy man (subject 1). The penis was in flaccid state. The upper and lower tracing are from the left and right sides of the base of the penis, respectively.

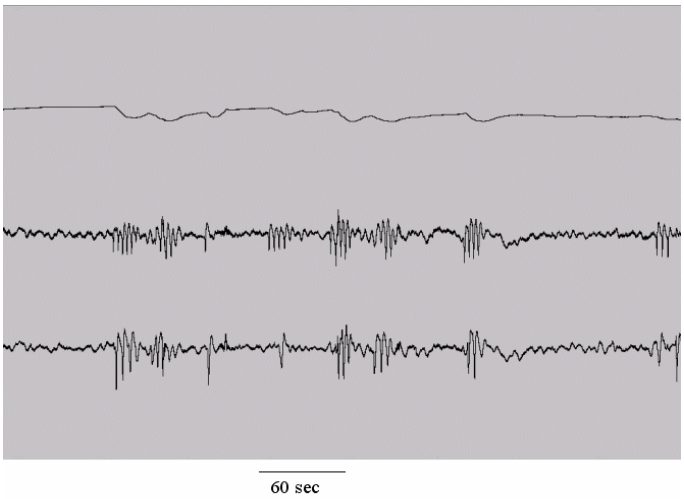

Figure 4. A part of a recording during a morning nap in a 22 years old healthy man (subject 11). The penis was in flaccid state. The set-up of the Barlow gauge and the electrodes are the same as Figure 1.

Discussion

In this study, sleep related erections occurred in 20 of the 22 (91%) recordings. This observation corroborates the notion that penile tumescence monitoring during morning naps is a valuable alternative for nocturnal monitoring in the assessment of erectile

function [9]. The chance that an erection occurs during a morning nap is similar to the chance that it occurs during nocturnal monitoring. However, the former is much easier to perform, less time-consuming and more cost-effective. Moreover, to study penile electrophysiology, sleep as a mediator of penile tumescence seems to be superior to audiovisually or pharmacologically mediated erection.

In accordance with the paradigm that CC-potentials mirror cavernous smooth muscle (CSM) contraction [2, 3] and that sympathetically mediated CSM contraction is inhibited during erection and increased during detumescence [10, 11], an absence of CC-potentials during tumescence and erection and continuous CC-potentials during detumescence are demonstrated. This finding is in sharp contrast with the literature, where even increases of frequency of CC-potentials (number of CC-potentials per time unit) during tumescence and rigidity have been described [4-7]. Apparently, in contrast to the sleep related erections assessed in this study, the condition in which the erectile response was triggered in the literature (audiovisually and pharmacologically) does not completely switch off sympathetically mediated activity (SMA). In these conditions SMA is probably sustained by nervousness, embarrassment and distraction. Nonetheless, as long as parasympathetic input is strong enough to overwhelm sympathetic activity, erection still occurs. During sleep, tumescence can be triggered by minimal parasympathetic activity in a period of sympathetic silence. Therefore, CC-EMG studies during sleep seem to be the best possible option to observe the changes in CC-SMA during natural erections without confounding psychological artifacts.

Another finding to support the notion that CC-potentials represent SMA of the CSM is the observation that CC-potentials are accompanied by penile shrinkage. This phenomenon has been described before in rabbits to occur in response to electrical stimulation of the sympathetic trunks [12].

Although, one would expect continuous CC-potentials during flaccidity, because the CC is kept in the flaccid state by constant (tonic) sympathetic activity [10, 11, 13]. However, in this study long periods of “electrical silence” interrupted by bursts of CC potentials

were observed. Apparently, even during "electrical silence", i.e. sympathetical silence, the CSM can maintain its contractile state. In isolated strips of bovine retractor penis muscle [14] and rabbit CC [15] both phasic and tonic contractions have been demonstrated during flaccidity. Phasic contractions are consistently associated with detectable electrical activity, whereas tonic contractions are not necessarily correlated with measurable electrical activity. Thus, tonic contractions do not necessarily go along with measurable electrical activity, probably because only a small volume of CSM cells is involved, and/or the activity of CSM cells is asynchronous, resulting in the summation of electrical activity too weak to be distinguished from baseline fluctuations by CC-EMG. Conversely, phasic contractions involve a large group of coordinated CSM cells generating synchronous electrical activity (CC-potentials). Therefore, we believe that tonic contractions represent the "resting" state of the CSM maintaining the basal tone, and phasic contractions are considered to be a response to internal and external stimuli, e.g. stretching of the CSM (due to an accumulation of blood), anxiety or a cold environment.

Conclusions

CC-EMG during morning naps is a practical and valid method to investigate the electrophysiology of the CC. With this set-up, we have shown that CC-potentials are absent during sleep related tumescence and erection and are increased during detumescence; during flaccidity penile shrinkage is observed accompanying CC-potentials. These results fit in the existing theory that CC-potentials reflect the SMA of the CSM.

Acknowledgements

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Chapter 5

Application of Correlation Techniques in the Analysis of Corpus Cavernosum Electromyographic Signals

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Submitted to J Urol

Abstract

Purpose: This study was aimed at establishing an objective, easy-to-use and comprehensive method to analyze corpus cavernosum (CC) electromyographic signals (CC-potentials).

Materials and methods: CC-potentials were recorded during flaccidity in 23 young healthy volunteers with surface electrodes placed on the penile shaft bilaterally. Based on the correlation function of Matlab software, an application program for the analysis of CC-potentials was developed. Individual CC-potentials and their autocorrelation function were evaluated, yielding parameters amplitude (*A*), duration (*D*), dominant frequency (*DF*), and number of dominant frequency periods (*DFP*). The cross-correlation function of both longitudinal and bilateral pairs of adjacent electrodes was calculated to assess the similarity and mutual delay of CC-potentials recorded simultaneously from different part of the CC. The parameters derived were squared maximum cross-correlation coefficient (*Rmax*) and delay (τ). Based on the absolute value of τ and the corresponding inter-electrode distance, propagation velocity (*PV*) was calculated.

Results: No significant difference related to the locations of the electrodes for parameters *A*, *D*, *DF*, and *DFP*, although the values of *A* and *DF* tended to be lower distally. The cross-correlation showed that both longitudinal and bilateral CC-potential pairs had highly similar shapes (the absolute values of *Rmax* were 0.80 ± 0.05 and 0.87 ± 0.06, respectively). The mean and standard deviation of *PV* were 6.15 ± 3.98 cm/sec.

Conclusions: The application program for correlation analysis of CC-potentials is a comprehensive and versatile method to analyze corpus cavernosum electromyographic recordings. Its objectiveness makes multi-center application possible.

Keywords: CC-EMG, corpus cavernosum, smooth muscle, electromyography, correlation

Introduction

Corpus cavernosum electromyography (CC-EMG) has failed to mature into a useful clinical tool, due to insufficient understanding of the electrophysiology of the corpus cavernosum (CC) and the recorded signals (CC-potentials), and a lack of standardization of the recording technique as well as signal processing and signal analysis methods [1]. Recently significant progress has been made to overcome these shortcomings. The methodology of CC-EMG recording was revisited. Monopolar recording has been shown to be superior to the traditional bipolar recording [2]. With this setup, further evidence has been obtained to support the notion that CC-potentials reflect sympathetically mediated electrical activity of cavernous smooth muscle (CSM) [2, 3]. However, a valid, objective, and easy-to-use method to analyze CC-potentials has not been established yet. Most clinical investigators measure the values of parameters manually [4-6], which is time consuming, imprecise and not objective. Stief et al. [7, 8] addressed this issue by introducing Fourier analysis and computerized classification of CC-potential components by fuzzy logic and neural networks. However, these methods have not been applied by other centers probably because the required basic knowledge of linear systems analysis is generally beyond the expertise of clinical physicians. Later on the same group explored the application of cross-correlation function to estimate time delay of CC-potentials recorded at different sites of the penis, which has only been published in a monograph (in German) [9] and did not result in the introduction of this method for the evaluation of CC-EMG recordings.

This study was aimed at establishing an analysis method that is easy-to-use for physicians, comprehensive, and objective. Because CC-potentials can be considered as spindle-like wave complexes [2], correlation techniques were used in a CC-EMG application program. This program was applied to a set of recordings in a group of young volunteers.

Materials and methods

Data collection

The methodology to record CC-potentials has been described in detail previously [2]. Briefly, with six or four surface electrodes (depending on the size of the penis) placed on the penile shaft bilaterally, CC-potentials were recorded simultaneously and monopolarly for 20-30 minutes during flaccidity. To allow monitoring the signals during the measurements, the recorded signals were digitized and filtered with a bandpass filter (cut-off frequencies 0.1 and 20.0 Hz). Both unfiltered and filtered digitized signals were stored on a computer. The longitudinal inter-electrode distances (center to center) were measured after completion of the measurements.

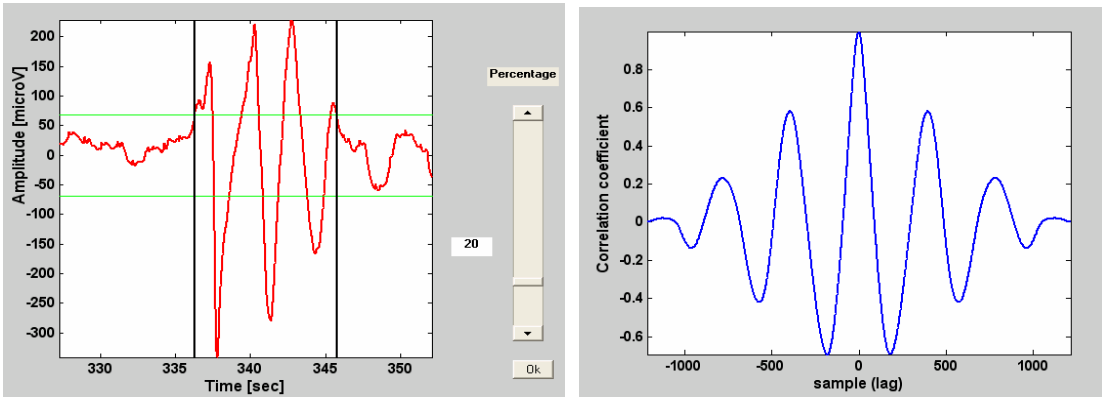
Using Matlab software (the MathWorks, Inc., Natick, Mass., USA), the unfiltered signals of each channel were extracted separately and filtered with a digital second-order bandpass filter (cut-off frequencies 0.1 and 5.0 Hz). The purpose of reducing the higher cut-off frequency to 5 Hz was to suppress high frequency, common signal components (in particular electrocardiographic signals) as much as possible without attenuating the CC-potential amplitude. It has been demonstrated that CC-potential power in normal subjects is actually below 5 Hz [2, 7].

CC- potential analysis

An application program for the analysis of CC-potentials was developed based on the correlation function of Matlab. Basically, the auto- and cross-correlation functions reduce the influence of noise, thus allowing the parameters of the CC-potential to be estimated more accurately than from the signal itself [10]. The onset and end of a CC-potential were set to correspond with oscillations exceeding a user-defined percentage of its maximum amplitude. After testing with different percentages, 20% turned out to be proper, because 20% of the maximum peak-to-peak amplitude was just above the baseline fluctuation (noise) level (around 75 μV) [6].

Original CC-potentials and their autocorrelation function were evaluated to characterize individual CC-potentials. In figures 1a and b a CC-potential and its autocorrelation function, respectively, are shown. CC-potentials were characterized by their amplitude

(*A*), duration (*D*), dominant frequency (*DF*), and number of dominant frequency periods (*DFP*). Different from the literature, *A* was defined as the voltage difference between the highest negative peak and the higher one of the two adjacent positive peaks. This strategy was chosen because measuring the voltage difference between the highest negative peak and the highest positive peak not adjacent to the negative peak may be markedly affected by baseline fluctuations when the baseline is instable (figure 2). *D* is the time window in seconds where the *A* of the CC-potential exceeds a user-defined percentage (20% in this study) of its maximum amplitude. *DF* (Hz), the frequency of a CC-potential where most signal power is present, was calculated from the time intervals between zero crossings of the autocorrelation function. *DFP* represents the number of *DF* periods of a CC-potential within *D* and is equal to the product of *D* and *DF*.



(a) (b)

Figure 1. (a) A CC-potential. The two horizontal lines indicate 20% of maximum amplitude of the *CC* potential; the two vertical lines indicate the onset and end of the CC-potential as determined automatically by the program. (b) Autocorrelation function of the CC-potential in figure 1 (a); lag is zero at highest positive peak (*Rmax* = 1).

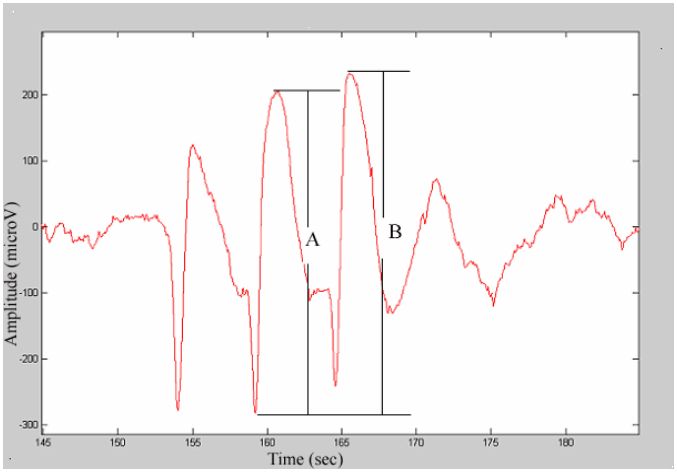


Figure2. The methods to determine amplitude. A: the voltage difference between the highest negative peak and the higher one of the two adjacent positive peaks, 491 μV. B: the voltage difference between the highest negative peak to the highest positive peak not adjacent to the negative peak, 531 μV. B is possibly affected by the baseline fluctuations superimposed to the CC-potentials.

To assess the similarity and mutual delay of CC-potentials recorded simultaneously from different sites of the corpora cavernosa, the cross-correlation function of both longitudinal and bilateral pairs of adjacent electrodes was calculated. Seven combinations were made for recordings with 6 electrodes (see figure 3), and 4 combinations in recordings with 4 electrodes. All visually identified CC-potentials with a typical spindle-like polyphasic waveform and a definite start and end were included, while CC-potentials disturbed by artifacts, monophasic, "incomplete" or long-lasting signals were excluded. In figures 4A and B, respectively, two simultaneously recorded CC-potentials and their cross-correlation function are shown. The parameters derived from the cross-correlation function are the squared maximum cross-correlation coefficient (*Rmax*) and the delay between two CC-potentials (τ). If the two signals are identical, then *Rmax* is 1; if they have no components in common, *Rmax* is 0; if they are identical but their phases are shifted by exactly 180^{o} (i.e. mirrored), then *Rmax* is -1. In order to allow a comparison of recordings with different numbers of CC-potentials, only data of five CC-potential pairs with the highest absolute values of *Rmax* were included. Based on the absolute value of τ and the corresponding inter-electrode distance, propagation velocity (*PV*) was estimated. To improve the accuracy, *PV* was calculated only from pairs of recordings with all 5, or 4

out of 5 selected CC-potential pairs having either a positive or a negative τ and thus the same "propagation" direction. When 4 out of 5 selected CC-potential pairs had the same sign of τ, only these values were included and the CC-potential pair with opposite sign of τ was omitted.

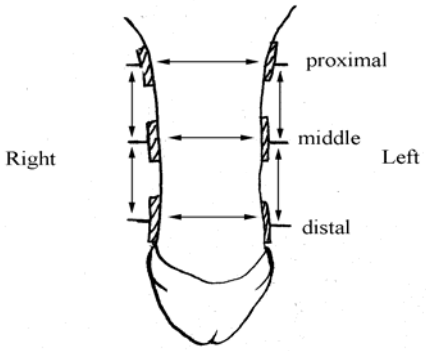
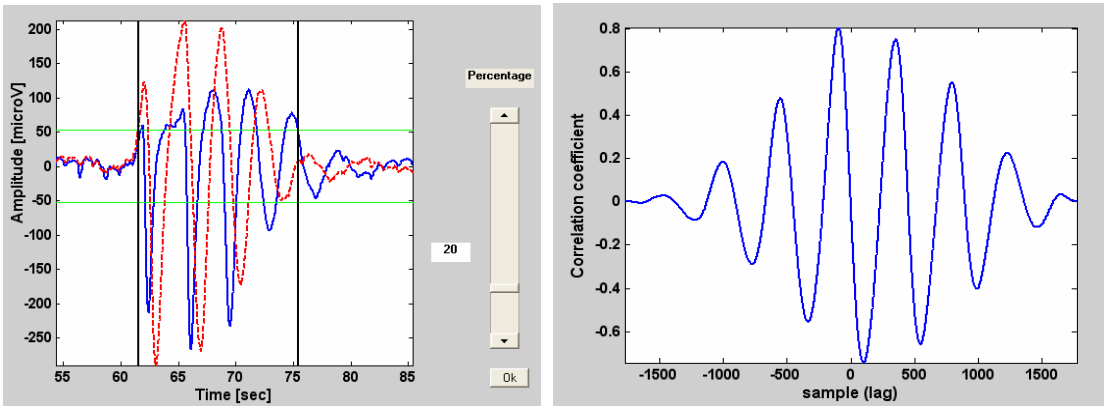


Figure 3. Electrode combinations for cross-correlation analysis. The arrows indicate the seven combinations in recordings using six electrodes



(a) (b)

Figure 4. (a) Two CC-potentials simultaneously recorded from the proximal (solid line) and middle (dashed line) sites of the CC. The two horizontal lines indicate 20% of maximum amplitude of the smaller *CC* potential; the two vertical lines indicate the start and end of the CC-potentials as determined automatically by the program. (b) Cross-correlation function of the CC-potential pairs in figure 2A. τ (+ or –) is determined from sample count corresponding to maximum closest to sample zero. *Rmax* = 0.81, τ = -0.73 sec.

In practice the cross-correlation analysis was performed first, in order to select five longitudinal CC-potential pairs with the highest absolute *Rmax* for the autocorrelation analysis.

Study population and recording equipment

Twenty-three healthy Caucasian men with a mean age of 24.7 (range 19 - 32) were included in this study. The volunteers were asked to refrain from alcohol, coffee, smoking and sexual activity within a period of 12 hours prior to the measurements. Informed consent was obtained from each subject.

Screener or a Porti system (TMS International, Enschede, The Netherlands) connected to a portable computer (Toshiba Satellitepro6100) was used to record CC-potentials. The amplifier parts of the two systems were identical. The two systems had a slightly different, fixed sampling rate (128 and 100 Hz, respectively). This difference does not affect the digitized CC-potentials, since the same filter was used to process the signals recorded with the two devices, and the low-pass cutoff frequency was only about 4-5% of the sampling rates. The electrodes were pre-gelled surface electrodes (type 9021S0231, Medtronic, Copenhagen, Denmark).

Statistical analysis

All data are given as mean ± standard deviation (SD) unless otherwise indicated. Two Factorial Analysis of Variance within the SPSS package was used to test the effect of electrode location (left and right side, proximal, middle and distal site) on parameters' values. The coefficient of variation (CV = (SD/mean) * 100%) was used to indicate the variability of τ, independent of the corresponding interelectrode distance. The CV of τ of longitudinal and bilateral CC-potential pairs was compared using Student's t test. A p value < 0.05 was regarded as significant.

Results

Among all 23 subjects, 10 had 6 electrodes and the other 13 had 4 electrodes. Five

electrode pairs showed nearly identical baseline fluctuations and CC-potentials (*Rmax* ≅ 1 and $\tau \cong 0$), indicating a short circuit between the corresponding adjacent electrodes. The CC-potentials recorded with these electrodes were excluded.

Single CC- potential analysis

The parameters *A*, *D*, *DF*, and *DFP* of CC-potentials recorded at the same level (proximal-middle-distal) but on opposite sides of the penis did not differ significantly (see table 1). Therefore, the values on both sides were taken together. The means and SDs of these parameters are shown in table 2. No significant difference related to the locations of the electrodes was found for any parameter, although the values of *A* and *DF* of the distal sites tended to be lower (table 1 and 2).

Table 1. Effects of electrode locations on CC-potential parameters (tested with Two Factorial Analysis of Variance)

		p value			
	Effect	*A*	*D*	*DF*	*DFP*
6 electrodes	Sides [#]	0.484	0.834	0.279	0.523
	Positions[$]	0.089	0.823	0.102	0.184
	Sides + positions	0.848	0.827	0.954	0.859
4 electrodes	Sides	0.451	0.739	0.543	0.457
	Positions	0.185	0.699	0.086	0.145
	Sides + positions	0.444	0.741	0.904	0.764

[#] Sides represent left and right sides

[$] Positions represent proximal, middle and distal sites

Table 2. Value of parameters *A*, *D*, *DF*, and *DFP* (data are given as mean (*SD*))

		n	*A* (μV)	*D* (sec)	*DF* (Hz)	*DFP*
6 electrodes	Proximal	9	378 (104)	12.34 (2.32)	0.28 (0.06)	3.46 (0.80)
	Middle	9	415 (99)	12.44 (3.14)	0.27 (0.06)	3.23 (0.67)
	Distal	9	333 (115)	12.97 (3.88)	0.24 (0.05)	3.02 (0.59)
4 electrodes	Proximal	13	342 (81)	13.04 (3.35)	0.24 (0.03)	3.08 (0.69)
	Distal	13	306 (92)	12.72 (2.63)	0.23 (0.03)	2.82 (0.50)

Analysis of CC- potential pairs

Data of 60 longitudinal and 47 bilateral electrode pairs were analyzed. The (absolute) values of *Rmax* of longitudinal and bilateral pairs were 0.80 ± 0.05 and 0.87 ± 0.06, respectively. The CV of the (absolute) values of τ of longitudinal and bilateral pairs

ranged from 7% to 132% (median 48%) and 22% to 150% (median 71%), respectively, and the variation of the latter was significantly larger compared to that of the former ($p < 0.001$). In tables 3A and B the "propagation" direction of longitudinal and bilateral CC-potential pairs are shown. In 71.7% of longitudinal pairs all 5 or 4 out of 5 CC-potential pairs propagated distally, and in 8.3% of them all 5 or 4 out of 5 CC-potential pairs propagated proximally. *PV* of longitudinal pairs of CC-potentials was estimated at 6.15 ± 3.98 cm/sec. The bilateral pairs had similar counts for a delay from left to right (27.7%) and in the opposite direction (29.8%).

Table 3. "Propagation" direction of longitudinal (A) and bilateral (B) CC-potential pairs

A

"Propagation" direction	Number of electrode pairs	Percentage
5 distally	34	56.7%
4 distally	9	15%
5 proximally	3	5%
4 proximally	2	3.3%
Other*	12	20%
Total	60	100%

B

"Propagation" direction	Number of electrode pairs	Percentage
5 left to right	10	21.3%
4 left to right	3	6.4%
5 right to left	8	17%
4 right to left	6	12.8%
Others*	20	42.6%
Total	47	100%

* 2 or more out of the 5 CC-potential pairs had a "propagation" direction different from the others

Discussion

In this study the correlation techniques are introduced as an easy-to-use, comprehensive, and objective method to analyze CC-potentials. In neurophysiology, correlation analysis is a well-established methodology to quantify the properties of striated muscle EMG and

other bioelectrical signals [10-12]. By yielding several parameters, the autocorrelation function allows for accurate characterization of individual bioelectric signals, such as CC-potentials. The cross-correlation function allows quantifying the similarity of simultaneously recorded signals (quantified as *Rmax*) and estimating their mutual time delay τ. If a signal, such as a CC-potential, is propagating between two electrodes, *PV* can be simply calculated [10, 11].

CC-potentials recorded with surface electrodes are supposed to reflect the superimposed oscillatory membrane currents of a group of adjoining CSM cells [1]. The contribution of these cells to the amplitude *A* of a CC-potential is inversely related to their distance from the recording electrode. When the electrical activity is centered under the electrode the CC-potential reaches its maximum *A*. Thus *A* of a CC-potential is proportional to the CSM content and the density of CSM cells contributing to the synchronized activity. Presumably, a decrease of *A* is expected when: (i) the CSM content is decreased (CSM degeneration), (ii) the thickness of the tissue enveloping CSM is increased (fat layer, edema or altered tunica albuginea, such as for example in Peyronie's disease), (iii) the intercellular communication via gap-junctions is impaired, (iiii) the sympathetic input is affected. The latter two situations may result in a diminished synchronization of the depolarization and repolarization of the CSM cell membranes. This presumption has been supported by the observation that the CC-potential *A* was decreased in patients with CSM degeneration [13], penile edema [14], diabetes mellitus [4], and in patients undergoing radical pelvic surgery, etc [5, 15].

Correlation analysis and spectral (Fourier) analysis of CC-potentials provide the same information in the time domain and the frequency domain, respectively. Accordingly, *DF* calculated from the autocorrelation function corresponds with the frequency with the highest power in the power density spectrum [7]. Since CC-potentials are supposed to reflect the superimposed membrane currents caused by Ca^{2+} influx through L-type Ca^{2+} channels of CSM cells [1], the value of *DF* as calculated in this study (around 0.25 Hz) is likely to correspond with the kinetics of these Ca^{2+} channels [16]. Theoretically, myogenic pathologies changing the membrane properties of CSM cells may alter the

value of *DF*.

The high *Rmax* of longitudinal pairs (0.80 ± 0.05) indicates that the waveforms of CC-potentials recorded simultaneously at different sites along the penile shaft are highly similar, although not identical. To have a high value of *Rmax*, two CC-potentials simultaneously recorded at different sites of a CC must meet the following conditions: (i) Two CC-potentials appear within a short time frame, i.e., they have a close temporal relation. (ii) Both CC-potentials have regular and similar waveforms reflected as similar *DF*, *DFP* and *D*. According to the existing knowledge, the sympathetic input and communication via gap junctions between CSM cells are responsible for the initiation, propagation and coordination of electrical activity within the CC [17, 18]. Based on this notion, sympathetic neuropathy or conditions affecting the communication between CSM cells may result in a decreased *Rmax* of CC-potential pairs by for example having irregular waveforms, different *DF*, *DFP* and *D*, or even a failure of propagating electrical activity from one site to another. Hence, in addition to other parameters, *Rmax* may be useful to detect sympathetic neuropathy and CSM dysfunction.

The majority of CC-potentials propagated in a distal direction, towards the tip of the CC (table 3A) indicating that CC-potentials are mostly initiated in the proximal part of the CC. However, in 38.3% of the longitudinal pairs the CC-potentials propagated both distally and proximally, suggesting that CC-potentials may be initiated at more than one site in the CC. In 5% of the pairs all 5 CC-potentials propagated in a proximal direction, suggesting that the initiation sites can be present in the distal part of the CC.

It is well known that the septum between the two human corpora cavernosa is incomplete, and spreading of ions/second messengers from one CC to another is possible [17]. Although the observation that bilateral CC-potential pairs had highly similar waveforms (high *Rmax*) and close temporal relation favors this notion, the facts that bilateral pairs had no preferential direction of τ and (table 3B), moreover, a much larger variation of τ (*CV* of τ of bilateral pairs are significantly larger compared to that of longitudinal pairs), suggest that the electrical interaction of the two corpora cavernosa, if

existing, is much weaker compared to that of proximal and distal parts of one CC in healthy men.

Although, a change in *PV is* an important indicator of muscle disease in striated muscle EMG [19], the value of *PV* to detect myogenic pathology in CC-EMG is questionable. At least two factors may limit the usefulness of *PV* in CC-EMG. Firstly, the accuracy of the calculated *PV* depends on the exact distance between electrode pairs. The inter-electrode distance, however, cannot be kept constant during a 20-30 min recording session (the penile length changes depending on a subject's sympathetic tone, room temperature, etc.). *PV* is only useful if its change caused by pathology is larger than the variation caused by the variable inter-electrode distance. Furthermore, *PV* can only be estimated correctly when the electrodes are aligned with the propagation direction. Because CSM is a three-dimensional structure, CC-potentials may propagate in various directions, even though the overall direction is longitudinally.

Although the clinical usefulness of *PV* is disputable, scientifically the findings of this study are helpful to increase our knowledge of the neurophysiology of the CC. We estimated that *PV* was 6.15 ± 3.98 cm/sec, whereas Gorek et al. calculated a similar mean value of 5 cm/sec [9]. This value indicates that CC-potentials travel via gap junctions among CSM cells rather than via nerve fibers. In the latter case, *PV* would have been much higher (around 100 cm/sec) [20].

In conclusion, the application program for correlation analysis of CC-potentials introduced in this study is a comprehensive and easy applicable method to analyze CC-EMG recordings. Its objectiveness makes multi-center application possible. By calculating the parameters *A*, *D*, *DF*, *DFP*, *Rmax*, and *PV*, CC-potentials can be characterized adequately. The next steps of our study are to test the reproducibility (stability) of the parameters, and to investigate their sensitivity and specificity to detect myo- and neurogenic pathology of the CC.

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Chapter 6

Intra-individual Reproducibility of the Corpus Cavernosum Electromyogram

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Abstract

Introduction: Although the corpus cavernosum electromyogram (CC-EMG) has been studied already for 17 years, doubts regarding its reproducibility have remained.

Aim: To assess the reproducibility of CC-EMG and the influence of confounding factors.

Methods: Three CC-EMG recording sessions were performed in 13 healthy young men under the same conditions. Furthermore, the effects of potentially confounding factors, such as intake of caffeine, alcohol and smoking, and sexual activity were investigated in the same population. Using auto- and cross-correlation techniques, CC-potentials were characterized with parameters amplitude (*A*), duration (*D*), dominant frequency (*DF*), number of *DF* periods (*DFP*), maximum cross-correlation coefficient of longitudinal and bilateral CC-potential pairs (*Rmax-lon.* and *Rmax-bi.*), and propagation velocity (*PV*).

Results: *DF*, *D*, *A* and *Rmax-lon.* showed significant correlations among three sessions; *DFP* and *PV* showed significant correlations between two sessions performed within the same day but not between those performed on different days; *Rmax-bi*. did not show significant correlations between any two sessions. Intake of caffeine, alcohol and smoking did not affect CC-potentials, while the recordings shortly after ejaculation showed a tendency of a less stable baseline and less CC-potentials with smaller *A*.

Conclusions: CC-potential parameters *DF*, *D*, *A*, and *Rmax-lon.* have been demonstrated to be reproducible. The results provide an essential basis for the application of CC-EMG as a tool for clinical and scientific purpose. CC-potentials are not sensitive to confounding factors such as intake of caffeine, alcohol and smoking, while measurements shortly after ejaculation should be avoided.

Key Words: CC-EMG, corpus cavernosum, electromyography, smooth muscle, penis, reproducibility

Introduction

Reproducibility is an essential prerequisite for using corpus cavernosum electromyography (CC-EMG) as a tool for diagnosing erectile dysfunction (ED) or investigating the physiology of the cavernous smooth muscle (CSM). Although CC-EMG has been studied already for 17 years [1-5], doubts regarding its reproducibility have remained. Notwithstanding that some studies have demonstrated that CC-potentials are reproducible [3, 6], their outcome may be questioned because it is based on visual inspection and not on quantitative evaluation. In the literature the only quantitative study on reproducibility of CC-EMG was done by Fabra et al. [4], who performed a comparison of two independent measurements in a group of healthy men, and demonstrated that most CC-potential parameters such as the number of CC-potentials per time unit at rest and after provocation maneuvers are irreproducible, while parameters such as amplitude and duration are reproducible.

To study the reproducibility of CC-potentials, at least three important conditions should be fulfilled. First, the recording methodology should be standardized. Second, the measurement conditions should be controlled because CC-potentials can be affected by a variety of internal and external factors. Finally, the parameters that are used to characterize CC-potentials should be exactly defined.

Recently, multichannel monopolar recording was identified as the optimal methodology for recording CC-EMG [7]. Based on this set-up correlation techniques were introduced to characterize CC-potentials, and a number of parameters were exactly defined (Jiang et al, submitted). In this study we set out to investigate the intra-individual reproducibility of these parameters by performing three recording sessions under well-defined conditions in a group of healthy potent young men. Furthermore, the effects of potentially confounding factors, such as intake of caffeine, alcohol, smoking and sexual activity were investigated.

Materials and Methods

Study Populations and Design

Thirteen healthy men with a mean age of 23.5 years (range 19 - 34) were included. Written informed consent was obtained from each subject. On day 1, the subject was asked to refrain from alcohol, coffee, smoking and sexual activity within a period of 12 hours prior to the measurements. The first and second recording sessions were performed between 8 and 12 AM on the same day, with an interval of 1 hour. After the second recording session, the subject was provided with a lunch. In addition, 7 subjects were asked to drink 200 ml coffee and 500 ml beer (5% alcohol), and to smoke if they had the habit. The other 6 subjects were asked to masturbate and ejaculate. The third CC-EMG measurement was performed within 30 minutes afterwards (after 1 PM). The fourth CC-EMG measurement was done under the same conditions as the first recording session 1 to 14 days after the first visit.

Equipment and Measurement Protocol

A Porti system (TMS International, Enschede, The Netherlands) connected to a portable computer (Toshiba Satellitepro6100) was used to record CC-EMG. The electrodes used were pre-gelled surface electrodes (type 9021S0231, Medtronic, Copenhagen, Denmark; 1.5 *2.0 cm in size).

Measurements were performed in a closed, quiet room with the examiner present. Room temperature was between 20 and 25 degree. The subject was in a supine position on an examination table and was asked to relax but not to sleep. Four electrodes were placed on the penile shaft bilaterally (2 at the base of the penis, and 2 close to the coronal sulcus). The reference electrode was placed on the kneecap, and the grounding electrode was placed on the thigh. The monopolar recordings started after 10-20 minutes equilibration, lasting 30 minutes during flaccidity. The signals were filtered using a bandpass filter (cut-off frequencies 0.1 and 20 Hz). Both unfiltered and filtered signals were digitized immediately and stored on the computer with a sampling rate of 100 Hz. The longitudinal inter-electrode distances (center to center) were measured after completion of the

measurements.

Evaluation and Analysis

First the recordings were evaluated globally by visual inspection. Attention was paid to the quality of the recording (e.g. the noise and occurrence of artifacts), the baseline characteristics, and the waveforms of CC-potentials. Second, quantitative analysis was done using the cross- and auto-correlation functions. To assess the similarity and mutual delay of CC-potentials recorded simultaneously from different parts of the corpora cavernosa, the cross-correlation functions of longitudinal (proximal vs. distal on both left and right sides) and bilateral (left vs. right at both proximal and distal sites) CC-potential pairs were calculated. From this function the maximum value of the cross-correlation coefficient (*Rmax-lon.* and *Rmax-bi*., respectively) and the time delay were determined. Those five CC-potential pairs with the highest absolute values of *Rmax* of each electrode combination were included. From the time delay and the corresponding inter-electrode distance the propagation velocity (*PV*) of longitudinal pairs was estimated. Next, five CC-potentials from either side of the penile base with the highest absolute *Rmax-lon.* were selected, and the corresponding values of the parameters amplitude (*A*), duration (*D*), dominant frequency (*DF*), and the number of dominant frequency periods (*DFP*) were determined from the original signals and their autocorrelation functions. The analysis methods and the definitions of the parameters have been described in detail elsewhere (Jiang et al, submitted).

For each recording session of each subject the mean of 10 values of the parameters *A*, *D*, *DF*, *DFP* and *PV* was calculated and the corresponding sets of mean values were used to calculate the correlation coefficient (r) between recording sessions 1 and 2 and 1 and 4, respectively, using the Pearson correlation analysis. A high r (varying between 0 and 1) indicates a high reproducibility. The mean parameter values of session 3 were compared to those of the same subjects in session 2, using the Paired Samples T-test. A p-value < 0.05 was considered statistically significant.

Results:

Typical spindle-like polyphasic CC-potentials were seen in all sessions of all subjects. However, the second, third and fourth recording sessions of one subject did not have enough CC-potentials (at least 5) for analysis. This subject belonged to the group with coffee and alcohol after the second session. Data from this subject were excluded. Another subject did not participate in the fourth recording session.

The recordings of session 1, 2 and 4 showed comparable global patterns and similar waveforms of *CC*-potentials by visual inspection. The patterns of CC-EMG before and after intake of caffeine, alcohol and smoking was comparable, while the recordings after ejaculation showed a tendency of a less stable baseline and less CC-potentials with smaller *A*.

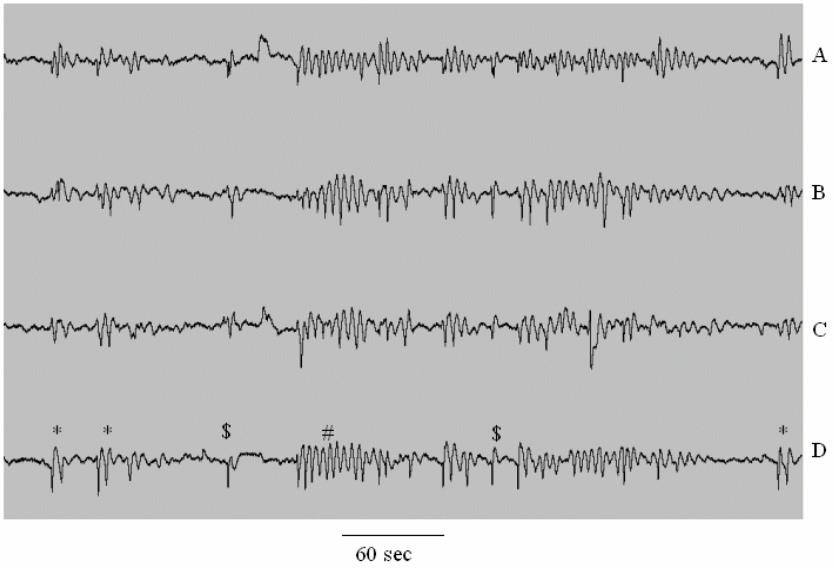


Figure 1. CC-EMG recordings in a 25 years old young man. A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. * indicates typical CC-potential, # indicates a long-lasting activity, and $ indicate "incomplete" signal.

Besides typical CC-potentials, often "incomplete" or long-lasting activity was recorded as well (Figure 1). Only CC-potentials with a typical spindle-like wave complex were included for quantitative analysis.

Table 1 shows the means and standard deviations of all parameters in recording sessions 1, 2 and 4. The r of each parameter (ranked according to their values) in sessions 1 vs. 2, and 1 vs. 4 are shown in table 2. In contrast to sessions 1 and 2 in which all parameters, except *Rmax-bi.*, were significantly correlated, sessions 1 and 4 had only a significant correlation of the parameters *DF*, *D*, *A* and *Rmax-lon*. In figure 2A-D regression lines of the latter four parameters are shown for session 1 vs. 2 and 1 vs. 4. No significant difference in any parameter was detected between sessions 2 and 3 in the subjects who drank coffee and alcohol (table 3A). After ejaculation, the value of *A* tended to be lower (table 3B).

Table 1. Mean values and standard deviations of each parameter in each of recording sessions 1, 2 and 4

	Rec. 1	Rec. 2	Rec. 4
A (μV)	340 ± 96	340 ± 49	362 ± 102
D (sec)	12.2 ± 2.5	12.7 ± 3.2	12.6 ± 2.6
DF (Hz)	0.26 ± 0.05	0.25 ± 0.04	0.27 ± 0.05
DFP	3.00 ± 0.62	3.02 ± 0.82	3.32 ± 0.52
Rmax-lon.	0.82 ± 0.06	0.83 ± 0.06	0.81 ± 0.04
Rmax-bi.	0.85 ± 0.05	0.88 ± 0.07	0.88 ± 0.05
PV (cm/sec)	5.1 ± 2.8	6.2 ± 3.6	4.5 ± 1.2

Table 2. Correlation coefficients (r) of parameters in recording sessions 1 vs. 2 and 1 vs. 4 in 12 healthy volunteers

	r (1 vs. 2)	r (1 vs. 4)	p (1 vs. 2)	p (1 vs. 4)
DF	0.85	0.88	0.00*	0.00*
D	0.71	0.91	0.01*	0.00*
A	0.68	0.92	0.01*	0.00*
Rmax-lon.	0.80	0.69	0.00*	0.02*
DFP	0.92	0.53	0.00*	0.10
PV	0.82	0.59	0.01*	0.10
Rmax-bi.	0.53	0.17	0.10	0.62

*Significant correlation

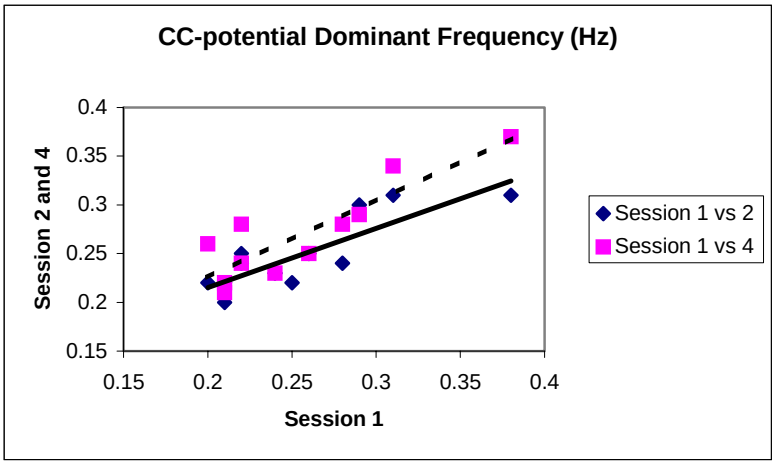
Table 3. Value of CC-potential parameters before and after intake of caffeine, alcohol and smoking (a) and ejaculation (b) (data are given as mean (*SD*), n = 6)

(a)

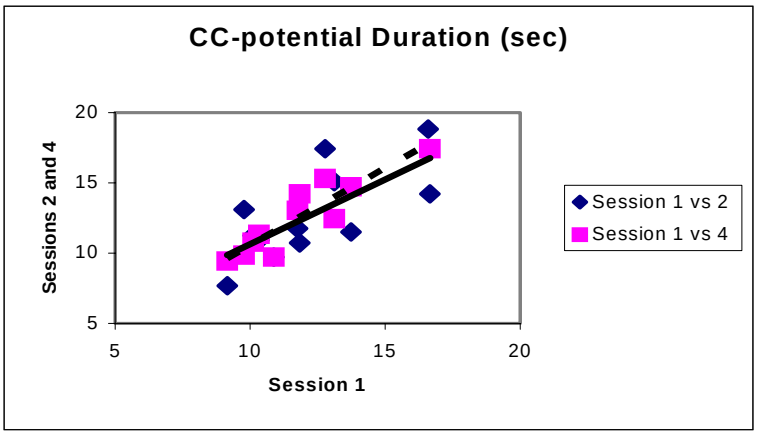
	Before	After	Paired difference (before – after)	p value
DF (Hz)	0.26 (0.04)	0.25 (0.04)	0.01 (0.01)	0.42
D (sec)	12.4 (3.7)	11.9 (2.98)	0.47 (2.87)	0.71
A (μV)	350 (48)	376 (98)	-26 (72)	0.42
Rmax-lon.	0.84 (0.05)	0.84 (0.04)	0.00 (0.03)	0.91

(b)

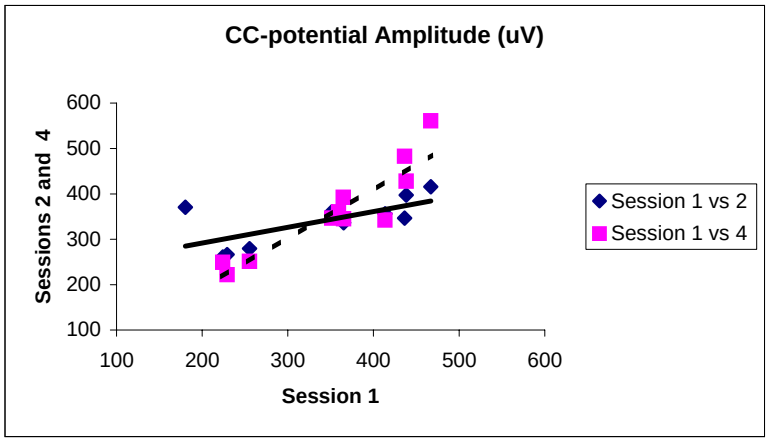
	Before	After	Paired difference (before – after)	p value
DF (Hz)	0.24 (0.03)	0.24 (0.04)	0.00 (0.03)	0.88
D (sec)	13.0 (3.0)	12.8 (2.2)	0.26 (3.27)	0.85
A (μV)	330 (51)	265 (136)	65 (101)	0.17
Rmax-lon.	0.82 (0.07)	0.82 (0.09)	0.01 (0.08)	0.88


CC-potential Dominant Frequency (Hz)
Session 2 and 4
0.4
0.35
0.3
0.25
0.2
0.15
0.15
0.2
0.25
0.3
0.35
0.4
Session 1
Session 1 vs 2
Session 1 vs 4

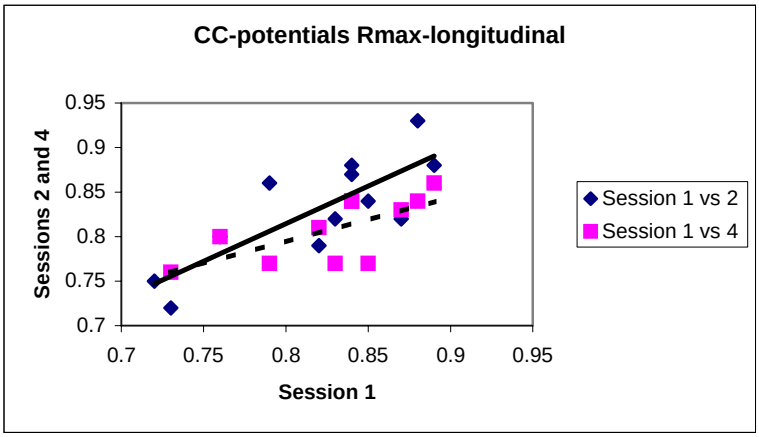
(a)


CC-potential Duration (sec)
Sessions 2 and 4
20
15
10
5
5
10
15
20
Session 1
Session 1 vs 2
Session 1 vs 4

(b)


CC-potential Amplitude (uV)
Sessions 2 and 4
600
500
400
300
200
100
100
200
300
400
500
600
Session 1
Session 1 vs 2
Session 1 vs 4

(c)



(d)

Figure 2. a-d show reproducibility of parameters dominant frequency (*DF*), duration (D), amplitude (*A*), and maximum cross-correlation coefficient of longitudinal CC-potential pairs (*Rmax-lon.*), respectively. In all these four parameters a significant correlation exists between both recording sessions 1 and 2 (solid line) and 1 and 4 (broken line).

Discussion

This study demonstrates that parameters such as dominant frequency (*DF*), duration (*D*), amplitude (*A*) and the maximum value of the cross-correlation coefficient of longitudinal CC-potential pairs (*Rmax-lon*.) are reproducible, whereas number of *DF* periods (*DFP*), propagation velocity (*PV*), and the maximum value of the cross-correlation coefficient of bilateral *CC*-potential pairs (*Rmax-bi.*) do not show sufficient reproducibility.

The underlying physiological mechanisms may help to understand why some parameters are reproducible whilst others are not. CC-potentials are supposed to reflect the superimposed membrane currents caused by Ca^{2+} influx through L-type Ca^{2+} channels of CSM cells [5, 8]. Therefore, DF is assumed to reflect the kinetics of these channels and may thus be dependent on the velocity of depolarization and repolarization of these cells. Next, *A* depends on the CSM content and the synchronization of electrical activity of adjoining CSM cells that varies with the sympathetic input and intercellular communication [2, 9]. Furthermore, *Rmax-lon.* quantifies the similarity of CC-potentials

recorded simultaneously at proximal and distal parts of the CC, and may, therefore, reflect the degree of coordination of electrical activity at different parts of the CC [10,11]. Although the physiological mechanism underlying *D* is unclear, all four reproducible parameters most likely reflect physiological properties of the CSM and its sympathetic innervation, which are supposed to be less susceptible to internal or external confounding factors. Furthermore, the mean values of these four parameters in three sessions are similar, and the values of *A* and *D* are comparable to those in the literature (table 1) [2-4]. These reproducible parameters may be regarded as the key parameters to characterize the CC-EMG.

Parameters *DFP* and *PV* appeared to be reproducible only between two recordings sessions performed within the same day with one hour in between, but irreproducible between sessions performed on different days. A possible explanation for this remarkable finding is that the variability of biological, technical, or environmental factors influencing the CC-EMG is greater when the interval between two recording sessions is longer. Furthermore, it should be considered that *DFP* is not an independent parameter because it is the product of *D* and *DF*. The irreproducibility may be due to the magnification of the variations of *D* and *DF*. *PV* is calculated as the interelectrode distance divided by the corresponding time delay. The interelectrode distance varies with the change in penile size depending on a subject's sympathetic tone, room temperature, etc., which makes *PV* variable.

Rmax-bi. quantifies the similarity of electrical activity from left and right corpora cavernosa. The irreproducibility of *Rmax-bi.* between two recording sessions conducted both within the same day and on different days may be because CC-potentials in the left and right corpora cavernosa are generated by independent processes, resulting in CC-potential pairs with a higher mutual variation than those in the same CC.

The number of CC-potentials per time unit was not assessed in this study, because we had already demonstrated in a previous study [7] that it is not reproducible, due to its dependency on the relaxation status (i.e., the sympathetic tone) of the subject [3, 7, 12],

which cannot be regulated voluntarily at a steady level. Furthermore, the frequent occurrence of "incomplete" or long-lasting CC activity, besides typical CC-potentials (figure 1) renders a reliable count of CC-potentials impossible.

As caffeine, alcohol, smoking and sexual activity are common ingredients of behavior, elucidating the impact of these factors on CC-potentials is of importance for the standardization of the measurement conditions. In this study we have shown that a moderate intake of caffeine, alcohol or smoking does not influence the features of CC-potentials. Furthermore, since the third recording was performed in the afternoon after lunch, it seems that eating and its timing does not affect CC-potentials either. A shortcoming of this study is that each factor was not tested separately in the small number of participants. Therefore, the possibility that counteracting effects exist cannot be ruled out. Interestingly, following ejaculation CC-EMG recordings showed a tendency of a less stable baseline and less CC-potentials with smaller amplitude. A possible explanation for this phenomenon is that the CSM and the sympathetic innervation are in a relatively "inactive" state in the resolution phase of the sexual response cycle, resulting in fewer and less synchronous activity. Because the parasympathetic system also is in a refractory state [13], the CC remains flaccid. The time needed for recovering basal level of cavernous electrical activity following ejaculation is unknown. Based on this finding the statement that recording CC-EMG shortly after ejaculation should be avoided seems justified.

Conclusions

We demonstrated that the CC-EMG parameters dominant frequency (*DF*), duration (*D*), amplitude (*A*), and maximum cross-correlation coefficient of longitudinal CC-potentials pairs (*Rmax-lon.*) are reproducible. This finding provides a basis for the application of CC-EMG as a clinical tool. Behavioral factors such as the consumption of coffee, alcohol and smoking seem not to influence cavernous electrical activity, whilst recording of CC-EMG shortly after sexual activity should be avoided.

Acknowledgements

The authors thank Jos Frantzen for his valuable comments, and John Philippi for his technical assistance.

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Chapter 7

Clinical Validation of Corpus Cavernosum Electromyography: a Study in 116 Patients with Erectile Dysfunction and 41 Potent Men

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To be submitted

Abstract

Objectives: to assess if corpus cavernosum electromyography (CC-EMG) parameters are age dependent, and if CC-EMG is discriminative for well defined clinical conditions.

Materials and methods: A total of 116 patients with erectile dysfunction (ED) and 41 potent men aged from 19 to 62 years were studied. ED patients were catalogued into six subgroups according to their etiologies: severe penile fibrosis, cavernous arterial insufficiency (CAI), vascular-risk factors (VRFs) without CAI, post radical retropubic prostatectomy (RRP), spinal cord lesions, and psychogenic ED. CC-EMG measurements were performed for 30 minutes during flaccidity. After evaluation by visual inspection, the recordings were analyzed using cross- and autocorrelation techniques. The parameters used were amplitude (*A*), duration (*D*), dominant frequency (*DF*) and maximum cross-correlation coefficient (*Rmax*) of CC-potentials recorded from proximal and distal parts of the CC.

Results: 13 out of the 14 patients with severe penile fibrosis did not show any distinguishable CC-potentials. No significant age dependence was detected in any parameters in potent men. Patients with CAI had significantly decreased *A* compared to patients with VRFs but without CAI and potent controls. Significantly decreased *A* and *Rmax* were detected in ED patients following RRP compared to the controls.

Conclusions: CC-potential parameters seem not age dependent. CC-EMG is able to discriminate ED patients with myo- or neuropathy from potent men. An absence of distinguishable CC-potentials or a significant decrease of CC-potential *A* may reflect cavernous smooth muscle degeneration, and a low *Rmax* can be regarded as a sign of autonomic neuropathy.

Keywords: CC-EMG, erectile dysfunction, corpus cavernosum, smooth muscle, electromyography

Introduction

Penile tumescence/erection and detumescence/flaccidity depend on synchronized relaxation and contraction of the cavernous smooth muscle (CSM), which is controlled by autonomic innervation [1]. Although it is generally accepted that the CSM and its autonomic innervation play key roles in penile erection, a robust non-invasive method to directly assess their functional state is not available. Penile biopsy [2] and a series of neurological tests [3, 4] have been introduced to serve this purpose. However, nowadays these methods are rarely applied by urologists, due to either its invasiveness or limited and unreliable information obtained [3, 5]. Corpus cavernosum electromyography (CC-EMG), a method being introduced in 1989 [6] and intensively investigated in the 1990's [5, 7-9], is regarded as a promising tool to directly evaluate the CSM and its autonomic innervation. Its noninvasiveness (using surface electrodes) makes it highly acceptable for both patients and physicians. Evidence from several centers indicates that CC-EMG is able to diagnose cavernous myo- and neuropathy in patients with ED caused by CSM degeneration, diabetes mellitus (DM), radical prostatectomy, and that it may play an important role in selecting candidates for invasive therapies [5-12]. However, its application as a diagnostic tool has never reached the level of the routine clinical practice due to a series of technical and practical difficulties [13]. One of its main defects is its lack of objective criteria to characterize CC-potentials. The often-appeared descriptions in the literature such as "normal"/ "abnormal", "regular"/ "irregular", or "synchronized"/ "desynchronized", etc. are rather vague, making a comparison of results from different centers difficult.

Recently, using a newly developed device, we have revised the recording methodology [14] (Chapter 3) and the signal analysis method (Chapter 5). Several parameters were exactly defined, which were proved to be reproducible (Chapter 6). In this study, we applied this new methodology in potent men of different age groups and ED patients with a variety of etiologies, aiming to assess if these parameters are age dependent, and if CC-EMG is discriminative for well defined clinical conditions.

Materials and methods

Study population

A total of 116 patients with ED and 41 potent men were studied. The patients presented at the outpatient departments of our hospitals with a complaint of ED. The potent men were either healthy young volunteers (n = 25, aged 19 – 34 years) or patients with prostate cancer waiting for a radical prostatectomy (n = 15, aged 44 – 62 years). The study was approved by the local ethical committee. Informed consent was obtained from each patient or volunteer.

The evaluation algorithm for ED patients consisted of a comprehensive medical, sexual and psychosocial history, physical examination, blood analysis, and penile-pharmaco duplex ultrasonography (PPDU). After these evaluations, the patients were catalogued into six subgroups according to their etiologies:

1. Intracavernous injection (ICI) therapy resistant patients due to severe penile fibrosis (n=14), including 6 patients following non-reversible low-flow priapsm, 5 patients received a penile prosthesis implant and had the prosthesis removed, 2 patients on long-term ICI therapy, not responding to that treatment anymore, and 1 patient with long-term DM and severe Peyronie's disease.
2. Patients with cavernous arterial insufficiency (CAI) (n=27). The diagnosis was based on PPDU. CAI was defined as an acceleration time equal to or higher than 100 ms [15]. Patients following pelvic surgery and those with proven peripheral or central neuropathy were excluded from this group.
3. Patients with vascular-risk factors (VRFs) but without CAI according to PPDU (n=22). VRFs included hypertension, coronary heart disease, hypercholesterolemia, and DM. Patients following pelvic surgery and those with proven peripheral or central neuropathy were excluded from this group.
4. Patients following radical retropubic prostatectomy (RRP) (n=19). All patients reported normal erectile function before the operation and ED afterwards. The interval between the operation and CC-EMG examination was at least 6 months.

5. Spinal cord (SC) lesions (n=9), including 8 patients with multiple sclerosis and 1 spinal cord injury patient undergoing Brindley's procedure.
6. Psychogenic ED (n=25). Patients who did not have any VRFs or co-morbidities which may cause organic ED, and who did not show any positive findings with physical examination, blood tests and PPDU were catalogued into this group.

Measurement protocol and signal analysis

The equipment, electrodes, and recording methodology of CC-EMG were described in detail before [14]. In brief, the measurements were done in a quiet, isolated room with the examiner present. With four surface electrodes placed on the penile shaft bilaterally, one reference electrode placed on the kneecap, and one ground electrode on the thigh, CC-EMG was performed monopolarly for 30 minutes during flaccidity. The digitized signals were stored on a computer for an offline review and analysis.

First, the recordings were evaluated globally by visual inspection. Attention was paid to the quality of the recording (e.g. the noise and occurrence of artifacts), the waveform of CC-potentials, and the number of typical CC-potentials. The goal of this step was to assess the suitability of the recordings for quantitative analysis. Only recordings with three or more CC-potentials during 30 minutes were included for quantitative analysis, because a reliable estimate of the parameters with only one or two CC-potentials is impossible. In recordings with more than five CC-potentials only the five CC-potential pairs with the highest absolute value of maximum cross-correlation coefficient (*Rmax*) of CC-potentials recorded at proximal and distal parts of the CC (described below) were included, to allow a comparison of recordings with different numbers of CC-potentials. Figure 1 shows an example of a recording suitable for quantitative analysis. Recordings with less then 3 potentials were excluded for quantitative analysis, but were divided into 3 subcategories: (1) Recordings with 1 or 2 CC-potentials. (2) Recordings without CC-potentials but only a straight line or slow baseline fluctuations. (3) Recordings with anarchic oscillations (Figure 2).

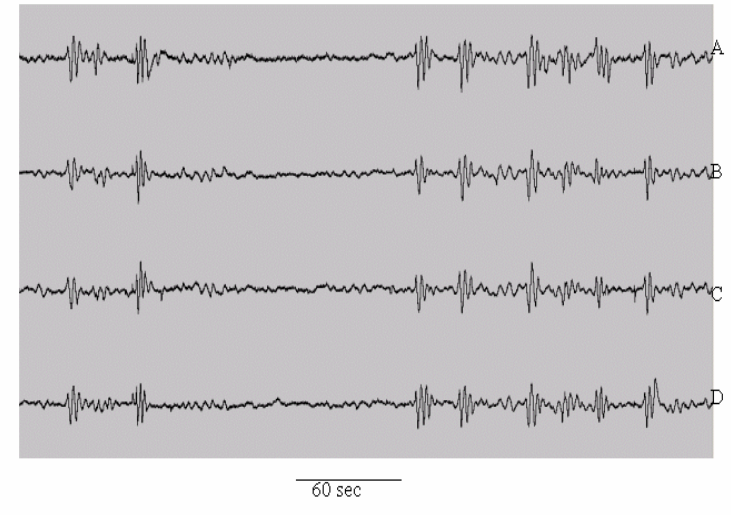

Figure 1. CC-EMG in a potent man. A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. CC-potentials with typical polyphasic spindle-like waveforms are seen. CC-potentials recorded at different parts of the CC are highly similar although not identical.

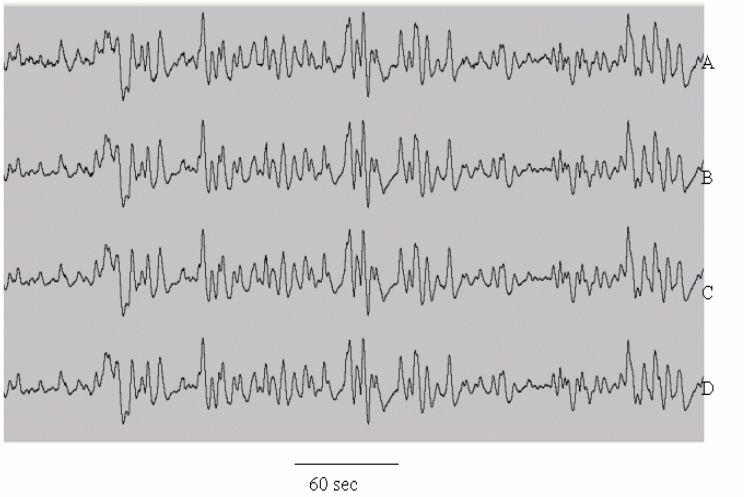

Figure 2. CC-EMG in a patient with VRFs. A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. No typical CC-potentials, but only anarchic oscillations can be seen.

Second, a quantitative analysis of CC-potentials was performed. The signal analysis method has been described in detail (Chapter 5). In short: to assess the similarity of the waveforms of CC-potentials recorded simultaneously from proximal and distal sites of

the CC, the cross-correlation function was calculated, which yielded the parameter *Rmax*. By calculating the autocorrelation function, the selected CC-potentials on both sides of the penile base were characterized by determining parameters amplitude (*A*), duration (*D*), and dominant frequency (*DF*).

Statistical analysis

Statistical methods used in this study included Pearson correlation analysis, Univariate Analysis of Variance, and LSD Post Hoc Test in ANOVA within the SPSS package. A p value < 0.05 was regarded as significant.

Results

Visual inspection of CC-potentials

The percentages of recordings suitable and not suitable for quantitative analysis in controls and different patient categories are shown in Table 1. The SC lesions, psychogenic ED and control groups had comparably high percentages of recordings with at least 3 CC-potentials (89, 92 and 90, respectively). This percentage was low in the penile fibrosis group: only 7. In the other three groups the percentages ranged from 63 to 74. 92.3% (13 out of 14) patients with penile fibrosis did not show CC-potentials, but only a straight line or slow baseline fluctuations (figure 3). This percentage was much higher than in the other groups. The CAI and post-RRP groups showed a relatively higher percentage of recordings without CC-potentials compared to other groups except the severe penile fibrosis group. The percentages of recordings with only 1 or 2 CC-potentials and anarchic recordings of different patient and control groups were comparable.

Table 1. Percentages of recordings suitable and not suitable for quantitative analysis in controls and different patient categories

	Suitable for quantitative analysais (≥ 3 potentiels)	Not suitable for quantitative analysis			
		1 or 2 potentials	No potentials	Anarchic	Total
Penile fibrosis	1 (7%)	0 (0%)	13 (93%)	0 (0%)	13 (93%)
CAI	20 (74%)	2 (7%)	4 (15%)	1 (4%)	7(26%)
VRFs	16 (73%)	5 (23%)	0 (0%)	1 (4%)	6(27%)
Post-RRP	12 (63%)	5 (26%)	2 (11%)	0 (0%)	7(37%)
SC lesions	8 (89%)	1 (11%)	0 (0%)	0 (0%)	1(9%)
Psychogenic ED	23 (92%)	1 (4%)	0 (0%)	1 (4%)	2(8%)
Controls	37 (90%)	3 (7%)	0 (0%)	1 (2%)	4(10%)
Total	117 (75%)	17 (11%)	19 (12%)	4 (3%)	40 (25%)

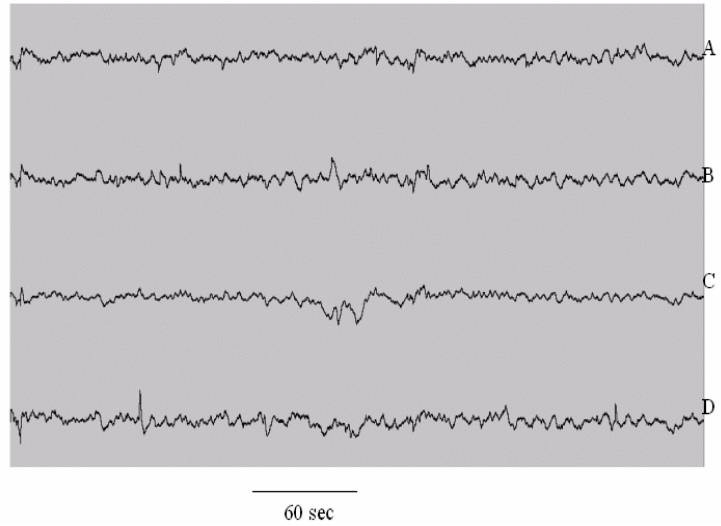

Figure 3. CC-EMG in a patient with penile fibrosis (post low-flow priapsm). A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. No typical CC-potentials but only slow oscillations can be seen.

Quantitative analysis of CC-potentials

In total 80 ED patients and 37 potent men with at least three CC-potentials were included for quantitative analysis.

Using Pearson Correlation analysis, no statistically significant age dependence was detected for any parameters (table 2A). Furthermore, the parameter values of young and

elderly potent men were compared using One-Way ANOVA. No significant difference was detected in any parameters, although *A* tended to be lower in elderly men (table 2B).

Table 2. A: correlation of parameter values versus age in 37 potent men; B: values of parameters in young and elderly potent men

A

	r	p value
A	-0.24	0.15
D	-0.06	0.73
DF	-0.29	0.08
Rmax	-0.13	0.45

B

	Young men (n = 24)	Elderly men (n = 13)	p value
A (μV)	355 ± 74	301 ± 88	0.06
D (sec)	12.8 ± 2.8	11.8 ± 2.1	0.26
DF (Hz)	0.26 ± 0.04	0.24 ± 0.04	0.16
Rmax	0.81 ± 0.05	0.80 ± 0.07	0.53

The inter-group differences of each parameter among the patient and control groups were assessed by Univariate Analysis of Variance using age as a covariate. No significant age effect was detected in any parameter. Significant inter-group differences existed in parameters *A* and *Rmax*, but not in *D* and *DF*.

To determine if the parameters *A* and *Rmax* differed significantly between the individual patient groups and the normal controls, LSD Post Hoc Test in ANOVA was performed. The estimated means after age corrections and 95% confidence intervals of *A* and *Rmax* in ED patients and potent men are shown in table 3 (a) and (b), respectively. A significantly lower *A* was detected in the CAI group compared to the control as well as the VRFs groups. A significant lower *A* and *Rmax* was detected in the post-RRP group compared to the control group. Figure 4 and 5 show recordings from a patient with CAI and a patient following RRP, respectively. The only penile fibrosis patient showing CC-potentials had markedly decreased *A* (202 μV).

Table 3. Means and 95% confidence intervals of parameters *A* (a) and *Rmax* (b) in each patient and control group. Data are given as means (95% confidence interval)

(a)

	n	*A* (μV)	p value compared to controls
CAI	20	269 (228-309)[#]	0.03[*]
VRFs	16	347 (304-389)	0.56
Post-RRP	12	254 (203-304)	0.02[*]
SC lesions	8	356 (297-415)	0.45
Psychogenic	23	349 (314-384)	0.45
Controls	37	331 (300-362)	--

*Significant

[#]Significant compared to the VRFs group (p = 0.01)

(b)

	n	*Rmax*	p value compared to controls
CAI	20	0.78 (0.74-0.82)	0.28
VRFs	16	0.77 (0.73-0.81)	0.12
Post-RRP	12	0.73 (0.68-0.77)	0.01[*]
SC lesions	8	0.79 (0.73-0.84)	0.50
Psychogenic	23	0.82 (0.79-0.85)	0.58
Controls	37	0.81 (0.78-0.84)	--

[*]Significant

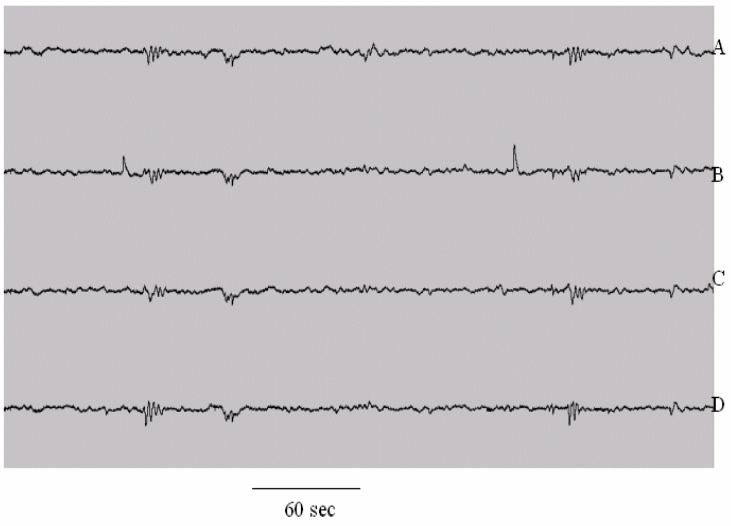

Figure 4. CC-EMG in a patient with DM and CAI. A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. The CC-potential *A* is decreased.

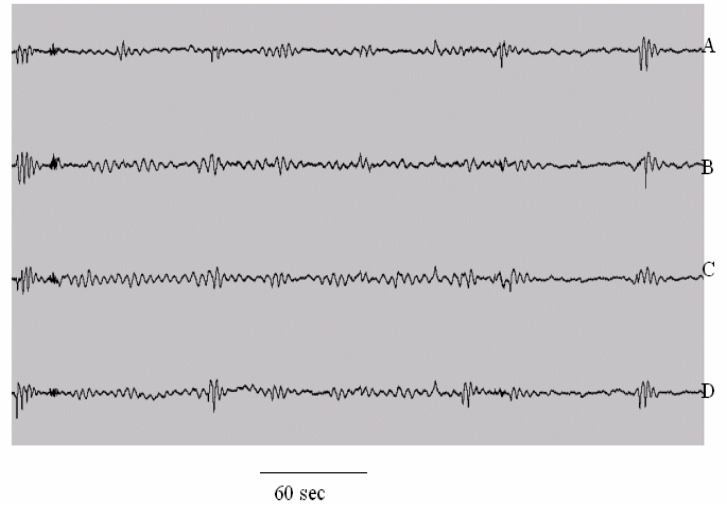

Figure 5. CC-EMG in a patient undergoing prostatectomy. A and B are from the left CC, from the proximal (A) and distal (B) electrodes; C and D are from the right CC, from distal (C) and proximal (D) electrodes. CC-potential *A* is lower, and the similarity (coordination) of electrical activity recorded at different parts of the CC is decreased.

Discussion

Following validation of the CC-EMG methodology and definition of reproducible parameters (*A*, *D*, *DF* and *Rmax*) in previous studies, we assessed the age dependence of these parameters and investigated their accuracy to confirm clinical diagnoses.

Knowing that clusters of CSM cells coordinated by the sympathetic nervous system are the source of the electrical activity recorded as CC-potentials [7, 8, 13] one would expect that specific clinical conditions associated with CSM loss and/or neuropathy go along with specific CC-EMG changes. A correlation between CSM content and *A* of CC-potential has been demonstrated before by Sattar et al [10]. Interestingly also in this study, *A* appeared to have discriminative power between clinical conditions. Moreover, *Rmax*, a parameter supposed to reflect coordination of the CC electrical activity seemed to be significantly lower in the post-RRP group. This finding supports the notions that (i) clinical conditions with loss of CSM cells go along with a decrease of *A* of CC-potentials and in a more severe state with undetectable electrical activity. And (ii) clinical conditions in which the innervation of the CC is disturbed go along with a lack of

coordination of the electrical activity, expressed by a decrease of *Rmax*. However, for parameters *D* and *DF* no significant difference between the clinical conditions was found. Therefore, these parameters seem not useful for a differential diagnosis.

Although it is well documented that aging goes along with a gradual fibrosis of the CC [2, 16], in this study no significantly lower *A* could be demonstrated in elderly men, which is in line with the literature [8, 9]. Several factors may contribute to this finding. First, the elderly volunteers in this study were selected to have a normal erectile function. Whereas, in general population the prevalence of ED in this age group is estimated to range between 10 and 20 percent [17, 18]. Second, the elderly men were relatively young (mean age: 56.9 years). And finally, the decline in *A* did not reach statistical significance may be because the number of subjects was relatively small.

A confirmation of the hypothesis that cavernous fibrosis goes along with a decrease of *A* followed in time by "electrical silence" as a kind of end-stage is found in the patients with severe fibrosis: 13 out of the 14 patients did not show any distinguishable CC-potentials. This was confirmed by Merckx et al. who showed that one post-priapism ED patient had no CC-potentials [11]. It should be noted that the absence of detectable CC-potentials does not represent a total "electrical silence", but as CC-potentials *A* too weak to be distinguished from the baseline fluctuations.

The other two groups, CAI and post-RRP, showed a relatively high percentage of recordings without CC-potentials (table 1). This finding can be explained by the fact that patients with CAI [19, 20] and patients following RRP [21] tend to develop CSM degeneration.

The reason that we separated recordings with 1 or 2 CC-potentials and recordings without distinguishable CC-potentials was that they may reflect different conditions. As discussed above, "electrical silence" may result from a severe decrease of CSM content, making the electrical activity too weak to be distinguished from the baseline fluctuations, while this is not the case in recordings with 1 or 2 CC-potentials with a normal *A*. Anarchic signals

(fig. 2) were observed in both ED patients and potent men. This has been observed before by several investigators [7, 8, 11, 14]. Anarchic signals are possibly caused by an elevated sympathetic tone due to stress, or a faulty connection of the electrodes, etc. [14].

The fact that patients with proven CAI showed a decreased *A* whereas men with VRFs but without CAI did not, seems to provide evidence for the concept that CAI is the end-stage of a process that gradually leads to severe cavernous fibrosis. Along that line of reasoning, CSM degeneration is a matter of time as penile (ultra)structure analyses have demonstrated before [19, 20]. The underlying mechanism could be that the chronic hypoperfusion of the cavernous tissue in patients with CAI induces the generation of tissue growth factor beta-1, a cytokine mediating the process of fibrosis, and consequently, causes penile fibrosis [22].

ED following RRP is most commonly caused by damage to the cavernous innervation during the operation. Additional damage to the arteries and secondary CSM degeneration due to denervation may contribute to ED [21]. In this study, conforming the literature [7, 11], patients following RRP had a low *Rmax* (corresponding to "desynchronization" of CC-potentials in the literature) and *A*. The decline of *A* may be the result of secondary CSM degeneration and/or desynchronization of electrical activity of CSM cells due to damage to autonomic innervation.

No significant change in any parameters was detected in the patients with SC lesions. The relatively small number of patients (n = 8) could be one reason. Furthermore, the location and extent of neurogenic damage of the patients were variable. Some patients might have an unaffected sympathetic pathway although the parasympathetic pathway was affected.

The finding that no difference in any parameters existed between the psychogenic ED and potent control groups fit well in our clinical expectations, since both the CSM and its autonomic innervation are supposed to be intact in this group of patients.

Although patients with CSM degeneration and those with autonomic neuropathy seem to have different CC-EMG patterns: only *A* but not *Rmax* is decreased in the former while both *A* and *Rmax* are decreased in the latter condition, sharply separating these two conditions is difficult or even impossible in a human study, due to the reasons that some clinical conditions impair both the autonomic nerves and CSM cells [23, 24], and disruption of the autonomic input can lead to secondary CSM degeneration [21, 25],

Much emphasis has been put on the role of CC-EMG in the evaluation of autonomic nerves in the literature [5-7]. However, the interpretation of neuropathy from a CC-EMG recording is more complicated compared to the evaluation of CSM degeneration. First, CC-EMG records electrical activity of CSM cells but not of the sympathetic nerves, although the former is controlled by the latter. On one hand, probably only limited or unreliable information about the sympathetic nerves could be obtained when the CSM is impaired; On the other hand, it is possible that some activity of the CC can still be recorded in case that the cavernous nerves are totally damaged, because CSM cells possess the ability of spontaneous activity [26]. Second, there is no a valid method to precisely determine neurogenic factors contributing to ED in the clinical practice [5]. And finally, since the sympathetic pathway originates from the eleventh thoracic to the second lumber spinal segments, while the parasympathetic pathway arises from the second, third and fourth sacral spinal cord segments [1], theoretically it is possible that patients with spinal cord lesions have normal sympathetically mediated activity, but impaired parasympathetically mediated activity, or verse visa.

Obviously, to overcome the above-mentioned problems more animal and clinical studies are needed. Animal models of pure CSM degeneration, peripheral nerve damage, low or high spinal cord injury, etc. can be specifically developed, enabling precisely correlate CC-EMG patterns to specific conditions. A previous study reported that CC-potential *A* decreased after excision of the cavernous nerves in rats [27]. In the future clinical study patients should be further cataloged according to the types and duration of VRFs, unilateral, bilateral or non-“nerve sparing” techniques during the RRP, and the location and extent of neuropathy, allowing more detailed interpretation of different CC-EMG

patterns. Furthermore, to define normative data and cutoff values of the parameters a larger number of patients and volunteers is required.

Conclusions

CC-potential parameters seem not age dependent. CC-EMG is able to discriminate ED patients with CSM degeneration or autonomic neuropathy from potent men. An absence of distinguishable CC-potentials or a significant decrease of CC-potential *A* may reflect CSM degeneration (penile fibrosis), and a low *Rmax* (reflecting the coordination of electrical activity at different parts of the CC) can be regarded as a sign of autonomic neuropathy.

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Chapter 8

Summary, Future Perspectives and Conclusions

Summary

The objective of this study was to develop corpus cavernosum electromyography (CC-EMG) into a useful clinical tool for evaluating the functional state of the cavernous smooth muscle (CSM) and its autonomic innervation.

In **chapter 2** we present an overview of the physiological background, the current status of CC-EMG, and discuss possibilities for further developments. Based on the existing knowledge, CC-potentials are supposed to reflect sympathetically medicated activity (SMA) of the CSM, and more specifically, the summation of membrane currents caused by Ca^{2+} influx through L-type voltage gated calcium channels of a group of CSM cells. We found that there is a lack of standardization of the devices, electrodes, signal recording, processing and analysis methodology of CC-EMG. Finally, in the literature promising results of clinical and animal studies can be found. However, results from different centers are difficult to be compared due to the lack of standardization.

In the first study (**chapter 3**) the methodology used for signal recording and signal processing was reestablished. Multichannel monopolar recording was demonstrated to have considerable advantages compared to the traditional 1 or 2 channel bipolar recording. Moreover, a band pass filter with cut-off frequencies of 0.1 and 20 Hz was found to be an optimal set-up for signal processing.

In the second study (**chapter 4**) in which CC-EMG was studied in the flaccid and erect state during morning naps, a consistent disappearance of CC-potentials during tumescence, and reappearance of continuous CC-potential oscillations during detumescence was observed. Moreover, penile shrinkage accompanied by CC-potentials was observed during flaccidity. These observations provide strong evidence to support the theory that CC-potentials reflect SMA of the CSM.

In the third study (**chapter 5**), correlation techniques were used to characterize individual CC-potentials and the mutual relation of CC-potentials recorded at different parts of the

CC. This methodology was demonstrated to be comprehensive, objective and versatile for analyzing CC-potentials.

In the fourth (**chapter 6**), the intra-individual reproducibility of CC-EMG was assessed. Parameters amplitude (*A*), duration (*D*), dominant frequency (*DF*) and maximum correlation-coefficient of longitudinal CC-potential pairs (*Rmax-lon.*) were proven to be reproducible and therefore, regarded as key parameters to characterize the CC-EMG.

And finally, in a clinical observation study (**chapter 7**) CC-EMG was applied in a group of potent volunteers in different age groups and ED patients with different clinical conditions. No significant age dependence in any parameter was detected in men with normal erectile function. Furthermore, CC-EMG was shown to be able to discriminate patients with clinical conditions that are associated with CSM degeneration and autonomic neuropathy.

Future perspectives

After addressing methodological and practical issues hindering the clinical application of CC-EMG in this thesis, still a number of basic questions about the initiation, propagation and coordination of the CC electrical activity as well as some clinical issues need to be clarified before CC-EMG can be used as a robust clinical tool.

For example, in vitro studies indicate that membrane currents caused by Ca^{2+} influx through L-type voltage gated calcium channels of CSM cells represents a source of CC-potentials. Convincing evidence from in vivo study is needed. Furthermore, the contribution of other ion currents to CC-potentials remains unclear.

With regard to the concept of coordination and propagation of electrical activity, the autonomic innervation and gap junctions are believed to play an important role. In this thesis some evidence was found that *Rmax* is decreased in a clinical condition where damage to the autonomic innervation is suspected (post radical prostatectomy). To make

a firm correlation between *Rmax* and coordination function of sympathetic input, studies in well defined specific models of unilateral and bilateral cavernous nerve damage, spinal cord injury at different levels are warranted. Furthermore, to date no experimental evidence regarding the influence of function of gap junctions on CC-potentials is available. Moreover, evidence is gained that conditions causing CSM degeneration go along with decrease of *A* and finally a total loss of detectable electrical activity. But also here robust proof is lacking. Whether or not a linear relation between CSM content and *A* exists needs to be clarified, which is difficult to be done in human studies since nowadays penile biopsy is no more feasible. Therefore, we believe that animal models are necessary to study these fundamental questions. Animal studies have the advantage that a variety of interventions can be employed, which are impossible in human studies.

Also more clinical studies are needed. Patients should be further cataloged according to for example the types and duration of vascular-risk factors, unilateral, bilateral or non-"nerve sparing" techniques during radical prostatectomy, and the location and extent of neuropathy, allowing more precisely interpretation of different CC-EMG patterns. Furthermore, to define normative data and cutoff values of the parameters a larger number of patients and volunteers is required.

Conclusions

1. Multichannel monopolar recording of CC-EMG is practical and has several advantages compared with traditional one or two channel bipolar recording.
2. CC-EMG during morning naps is a practical and valid method to investigate the electrophysiology of the CC. The patterns of CC-EMG signals during tumescence, detumescence and flaccidity fit in the existing theory that CC-potentials reflect SMA of the CSM.
3. The application program for correlation analysis of CC-potentials is a comprehensive, objective and versatile method to analyze CC-EMG recordings.

4. Parameters *DF*, *D*, *A*, and *Rmax-lon.* have been demonstrated to be reproducible intra-individually and therefore, they may be regarded as the key parameters to characterize the CC-EMG.
5. CC-potential parameters seem not age dependent. CC-EMG is able to discriminate ED patients with CSM degeneration or autonomic neuropathy from potent men.

Samenvatting

Corpus Cavernosum Elektromyografie (CC-EMG) in de diagnostiek van erectiele disfunctie

Doel van dit onderzoek is om corpus cavernosum electromyografie(CC-EMG), een methode om de functie van het peniele zwellichaam (corpus cavernosum) te onderzoeken aan de hand van door het gladdespier weefsel opgewekte elektrische potentialen, te ontwikkelen tot een klinisch toepasbare methode. CC-EMG werd in 1989 voor het eerste beschreven door Gorm Wagner, maar werd, ondanks het feit dat het een veelbelovende methode leek, om verschillende redenen klinisch nooit op brede schaal toegepast.

In **hoofdstuk 2** worden aan de hand van een literatuuronderzoek 1)een overzicht gegeven van de (elektro)fysiologie van het corpus cavernosum (CC), 2) de problemen waarop de klinische toepassing van het CC-EMG tot dan toe was gestuit en 3) de mogelijke oplossingsrichtingen besproken. De elektrische activiteit die in het zwellichaam in de vorm van actiepotentialen wordt opgewekt wordt beschouwd als een weerspiegeling van de door het sympatische zenuwstel gecoördineerde contractie van groepjes caverneuze (gladde)spiercellen die met elkaar communiceren door middel van gap-junctions. De actiepotentialen zijn een optelsom van veranderingen in membraan spanning van deze groepjes gladde spiercellen. De potentialen ontstaan doordat Ca^{2+} ionen door zogenaamde L-type voltage gated calcium kanalen de cel instromen. De gebrekkige standaardisatie van CC-EMG apparatuur, elektroden en methoden van meten en analyseren van de gemeten elektrische activiteit (waardoor ieder centrum dat zich met CCEMG bezighield andere resultaten boekte) bleken de voornaamste redenen te zijn voor het geringe succes van CC-EMG in de kliniek. Bovendien was eind jaren 90 de behoefte aan een methode om de exacte oorzaak van een erectiestoornis vast te stellen verdwenen door de introductie van erectieondersteunende medicatie die ongeacht de oorzaak voor elk type ED werkzaam was.

In **hoofdstuk 3** wordt gezocht naar meest geschikte manier om de elektrische activiteit van het zwellichaam te registreren en te verwerken. Monopolaire registratie via minimaal

4 huidelektroden blijkt aanzienlijke voordelen te bieden boven de traditionele registraties met 1 of 2 bipolaire huid- of naaldelektroden. Voor de verwerking van de signalen blijkt een filterinstelling met een laagste afkap frequentie van 0.1 Hz en een hoogste afkap frequentie van 20 Hz de meest optimale.

In **Hoofdstuk 4** wordt het CC-EMG bestudeerd in de slappe en erecte penis tijdens ochtendslaapjes in het laboratorium. Zoals verwacht, verdwijnen de potentialen gedurende het stijf worden van de penis en worden continue oscillerende potentialen waargenomen bij het verslappen van de penis. Bovendien blijkt dat de penis in slappe toestand nog verder krimpt wanneer potentialen worden waargenomen. Deze observaties zijn een verdere ondersteuning voor de hypothese dat CC-potentialen de contractie van gladspierweefsel in het zwellichaam weerspiegelen.

In **Hoofdstuk 5** worden correlatietechnieken toegepast om individuele potentialen en de samenhang van de op verschillende plaatsen op het CC gemeten potentialen te karakteriseren. Aangetoond wordt dat de toepassing van deze technieken een goede mogelijkheid biedt om de verschillende parameters van het CC-EMG te karakteriseren.

Op grond van de in hoofdstuk 5 gekarakteriseerde parameters (Amplitudo, Duur, Dominante Frequentie en correlatiecoëfficiënt van twee longitudinale potentiaal paren) wordt in **Hoofdstuk 6** de intra-individuele reproduceerbaarheid van CC-EMG onderzocht. Uit dit onderzoek blijkt dat de parameters reproduceerbaar zijn en dus gebruikt kunnen worden om het CC-EMG te karakteriseren.

Tot slot wordt in **Hoofdstuk 7** in een observationele klinische studie CC-EMG toegepast in groep normale vrijwilligers van verschillende leeftijd en mannen met erectiestoornissen met verschillende oorzaken. In dit onderzoek werden geen aanwijzingen gevonden dat CC-EMG leeftijdsafhankelijk is. Bovendien lijken degeneratieve aandoeningen van CC en autonome neuropathie gepaard te gaan met een afwijkend CC-EMG.

概括

虽然勃起功能障碍的诊治在近２０年中取得了长足进展，目前临床上尚缺乏一种有效的和无创伤性的评估阴茎海绵体平滑肌和其自主神经支配的方法。自１９８９年被第一次报道以来，阴茎海绵体肌电图被认为是能满足这一临床需要的最有希望的检查方法。然而，一些技术上和应用上的困难阻碍了这一方法的广泛临床应用。本研究的目的是最终将阴茎海绵体肌电图发展成为检测阴茎海绵体平滑肌和其自主神经支配的功能状态的有用的临床诊断工具。

在本论文的**第二章**中我们综述了有关阴茎海绵体肌电图的基础生理知识和当前研究情况，并展望进一步研究的可能性。根据目前的知识，阴茎海绵体电位被认为反映了阴茎海绵体平滑肌的交感神经介导的电活动。更进一步说，其反映了一组阴茎海绵体平滑肌细胞的由钙离子经过细胞膜上L型电压控制型钙通道内流所引起的膜电流的总和。目前在阴茎海绵体肌电图的研究中尚缺乏关于记录设备、电极，以及信号记录，处理和分析方法的统一标准。虽然文献中报道了一些关于其临床和基础研究的有意义的结果，由于缺乏统一标准，来自不同中心的结果难于互相比较。

在**第三章**中我们重建了阴茎海绵体肌电图的信号记录和信号处理方法。多导单极记录法被证明优于传统的一或二导双极记录法。此外，信号处理所用的带通滤波器的截止频率被设定为0.1 和20 赫兹。

第四章研究了凌晨睡眠过程中在阴茎勃起和非勃起状态下阴茎海绵体肌电图的表现。在阴茎勃起过程中阴茎海绵体电位完全消失，而在勃起消退过程中出现了增强的持续的海绵体电活动。在非勃起状态中，伴随阴茎海绵体电位的出现，阴茎收缩活动被观察到。这些结果提供了有力证据支持阴茎海绵体电位反映了阴茎海绵体平滑肌交感神经介导的电活动的理论。

在**第五章**中自相关和互相关技术被应用于分析独立阴茎海绵体电位的特征和记录于不同部位的阴茎海绵体电位间的相互关系。这些方法被证明可以全面，客观和有效地分析阴茎海绵体电位。

在**第六章**中我们评估了阴茎海绵体肌电图的自体间重复性。阴茎海绵体电位的参量 - - 振幅，持续时间，频率，以及纵向阴茎海绵体电位对的最大互相关系数被证明为具有可重复性，因此，被用为分析阴茎海绵体肌电图的关键参量。

最后在**第七章**我们对处于不同年龄组的具有正常勃起功能的志愿者和由不同临床情况导致的勃起功能障碍患者进行了阴茎海绵体肌电图研究。在具有正常勃起功能的志愿者中所有参量均未表现出显著年龄依赖性。在勃起功能障碍患者中我们发现阴茎海绵体肌电图可以鉴别阴茎海绵体平滑肌退化（纤维化）和自主神经病变。

总之，本研究重建了阴茎海绵体肌电图的信号记录和处理方法，增进了对阴茎海绵体肌电图信号的生理意义的理解，建立了一套客观全面的信号分析方法，并定义了一些具有重复性的参量。最后在临床研究中我们发现应用这些参量可以诊断阴茎海绵体的平滑肌和自主神经病变。然而在阴茎海绵体肌电图最终能被广泛应用于临床之前，一些关于阴茎海绵体电生理的基础问题和诊断勃起功能障碍的具体临床问题还有待解 决。这些都将是我们下一步研究的计划。

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Curriculum Vitae

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Publications of the author

International publications:

1 **Jiang X**, Meuleman EJH, Wijkstra H, Wagner G. Corpus Cavernosum Electromyography during Morning Naps in Healthy Volunteers. Further Evidence that CC-potentials Reflect Sympathetically Mediated Activity. J Urol 2005; 174:1917-1920.

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